

to grow at 37 °C until an OD 600 of about 0.5 is reached. Take out an aliquot as T0 sample. Add 1 mM IPTG and allow to grow at 30 °C for 3 hours. Spin down the cells and store at -80 °C until purification. The determined cDNA and amino acid sequences for the P510S-C construct are shown in SEQ ID NO: 823 and 826, respectively.

5 The predicted third extracellular domain of P510S (P510S-E3; residues 328-676 of SEQ ID NO: 538) was expressed in *E. coli* as follows. The P510S fragment was amplified by PCR using the primers shown in SEQ ID NO: 830 and 831. The primer of SEQ ID NO: 830 is a sense primer with an NdeI site for use in ligating into pPDM. The primer of SEQ ID NO: 831 is an antisense primer with an added XhoI site
10 for use in ligating into pPDM. The resulting fragment was cloned to pPDM at the NdeI and XhoI sites. Clones were confirmed by sequencing. For protein expression, the clone was transformed into *E. coli* BL21 (DE3) CodonPlus-RIL competent cells. After induction, an OD600 of greater than 2.0 was achieved after 3 hours. Coomassie stained SDS-PAGE showed an over-expressed band at about 39 kD, and N-terminal sequencing
15 confirmed the N-terminal to be that of P510S-E3. Optimized culture conditions are as follows: dilute overnight culture/daytime culture (LB + kanamycin + chloramphenicol) into 2x YT (kanamycin and chloramphenicol) at a ratio of 25 ml culture to 1 liter 2x YT. Allow to grow at 37 °C until OD 600 equals 0.6. Take out an aliquot as T0 sample. Add 1 mM IPTG and allow to grow at 30 °C for 3 hours. Take out a T3
20 sample, spin down the cells and store at -80 °C until purification. The determined cDNA and amino acid sequences for the P510S-E3 construct are provided in SEQ ID NO: 824 and 827, respectively.

g) Expression of P775S in *E. Coli*

25 The antigen P775P contains multiple open reading frames (ORF). The third ORF, encoding the protein of SEQ ID NO: 483, has the best motif score. An expression fusion construct containing the *M. tuberculosis* antigen Ra12 (SEQ ID NO: 819) and P775P-ORF3 with an N-terminal 6x HisTag was prepared as follows. P775P-ORF3 was amplified using the sense PCR primers of SEQ ID NO: 832 and the anti-sense PCR primer of SEQ ID NO: 833. The PCR amplified fragment of P775P and

Ra12/pCRX1 were digested with the restriction enzymes EcoRI and XhoI. Vector and insert were ligated and then transformed into NovaBlue cells. Colonies were randomly screened for insert and then sequenced. A clone having the desired sequence was transformed into *E. coli* BL21 (DE3) CodonPlus-RIL competent cells. Two hours after
5 induction, the cell density peaked at OD600 of approximately 1.8. Coomassie stained SDS-PAGE showed an over-expressed band at about 31 kD. Western blot using 6x HisTag antibody confirmed that the band was Ra12-P775P-ORF3. The determined cDNA and amino acid sequences for the fusion construct are provided in SEQ ID NO: 834 and 835, respectively.

10

H) Expression of a P703P His tag fusion protein in *E. coli*

The cDNA for the coding region of P703P was prepared by PCR using the primers of SEQ ID NO: 836 and 837. The PCR product was digested with EcoRI restriction enzyme, gel purified and cloned into a modified pET28 vector with a His tag
15 in frame, which had been digested with Eco72I and EcoRI restriction enzymes. The correct construct was confirmed by DNA sequence analysis and then transformed into *E. coli* BL21 (DE3) pLys S expression host cells. The determined amino acid and cDNA sequences for the expressed recombinant P703P are provided in SEQ ID NO: 838 and 839, respectively.

20

I) Expression of a P705P His tag fusion protein in *E. coli*

The cDNA for the coding region of P705P was prepared by PCR using the primers of SEQ ID NO: 840 and 841. The PCR product was digested with EcoRI restriction enzyme, gel purified and cloned into a modified pET28 vector with a His tag
25 in frame, which had been digested with Eco72I and EcoRI restriction enzymes. The correct construct was confirmed by DNA sequence analysis and then transformed into *E. coli* BL21 (DE3) pLys S and BL21 (DE3) CodonPlus expression host cells. The determined amino acid and cDNA sequences for the expressed recombinant P705P are provided in SEQ ID NO: 842 and 843, respectively.

30

J) Expression of a P711P His tag fusion protein in *E. coli*

The cDNA for the coding region of P711P was prepared by PCR using the primers of SEQ ID NO: 844 and 845. The PCR product was digested with EcoRI restriction enzyme, gel purified and cloned into a modified pET28 vector with a His tag in frame, which had been digested with Eco72I and EcoRI restriction enzymes. The correct construct was confirmed by DNA sequence analysis and then transformed into *E. coli* BL21 (DE3) pLys S and BL21 (DE3) CodonPlus expression host cells. The determined amino acid and cDNA sequences for the expressed recombinant P711P are provided in SEQ ID NO: 846 and 847, respectively.

K) Expression of P767P in *E. coli*

The full-length coding region of P767P (amino acids 2-374 of SEQ ID NO: 590) was amplified by PCR using the primers PDM-468 and PDM-469 (SEQ ID NO: 935 and 936, respectively). DNA amplification was performed using 10 µl 10X Pfu buffer, 1 µl 10 mM dNTPs, 2 µl each of the PCR primers at 10 µM concentration, 83 µl water, 1.5 µl Pfu DNA polymerase (Stratagene, La Jolla, CA) and 1 µl DNA at 100 ng/µl. Denaturation at 96°C was performed for 2 min, followed by 40 cycles of 96°C for 20 sec, 66°C for 15 sec and by 72°C for 2 min., and lastly by 1 cycle of 72°C for 4 min. The PCR product was digested with XhoI and cloned into a modified pET28 vector with a histidine tag in frame on the 5' end that was digested with Eco72I and XhoI. The construct was confirmed to be correct through sequence analysis and transformed into *E. coli* BL21 pLysS and BL21 CodonPlus RP cells. The cDNA coding region for the recombinant B767P protein is provided in SEQ ID NO: 938, with the corresponding amino acid sequence being provided in SEQ ID NO: 941. The full-length P767P did not express at high enough levels for detection or purification.

A truncated coding region of P767P (referred to as B767P-B; amino acids 47-374 of SEQ ID NO: 590) was amplified by PCR using the primers PDM-573 and PDM-469 (SEQ ID NO: 937 and 936, respectively) and the PCR conditions described above for full-length P767P. The PCR product was digested with XhoI and cloned into the modified pET28 vector that was digested with Eco72I and XhoI. The

construct was confirmed to be correct through sequence analysis and transformed into *E. coli* BL21 pLysS and BL21 CodonPlus RP cells. The protein was found to be expressed in the inclusion body pellet. The coding region for the expressed B767P-B protein is provided in SEQ ID NO: 939, with the corresponding amino acid sequence
5 being provided in SEQ ID NO: 940.

EXAMPLE 18

PREPARATION AND CHARACTERIZATION OF ANTIBODIES AGAINST PROSTATE-SPECIFIC POLYPEPTIDES

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a) Preparation and Characterization of Polyclonal Antibodies against P703P, P504S and P509S

Polyclonal antibodies against P703P, P504S and P509S were prepared as follows.

15 Each prostate tumor antigen expressed in an *E. coli* recombinant expression system was grown overnight in LB broth with the appropriate antibiotics at 37°C in a shaking incubator. The next morning, 10 ml of the overnight culture was added to 500 ml to 2x YT plus appropriate antibiotics in a 2L-baffled Erlenmeyer flask. When the Optical Density (at 560 nm) of the culture reached 0.4-0.6, the cells were
20 induced with IPTG (1 mM). Four hours after induction with IPTG, the cells were harvested by centrifugation. The cells were then washed with phosphate buffered saline and centrifuged again. The supernatant was discarded and the cells were either frozen for future use or immediately processed. Twenty ml of lysis buffer was added to the cell pellets and vortexed. To break open the *E. coli* cells, this mixture was then run
25 through the French Press at a pressure of 16,000 psi. The cells were then centrifuged again and the supernatant and pellet were checked by SDS-PAGE for the partitioning of the recombinant protein. For proteins that localized to the cell pellet, the pellet was resuspended in 10 mM Tris pH 8.0, 1% CHAPS and the inclusion body pellet was washed and centrifuged again. This procedure was repeated twice more. The washed

inclusion body pellet was solubilized with either 8 M urea or 6 M guanidine HCl containing 10 mM Tris pH 8.0 plus 10 mM imidazole. The solubilized protein was added to 5 ml of nickel-chelate resin (Qiagen) and incubated for 45 min to 1 hour at room temperature with continuous agitation. After incubation, the resin and protein mixture were poured through a disposable column and the flow through was collected. The column was then washed with 10-20 column volumes of the solubilization buffer. The antigen was then eluted from the column using 8M urea, 10 mM Tris pH 8.0 and 300 mM imidazole and collected in 3 ml fractions. A SDS-PAGE gel was run to determine which fractions to pool for further purification.

As a final purification step, a strong anion exchange resin such as HiPrepQ (Biorad) was equilibrated with the appropriate buffer and the pooled fractions from above were loaded onto the column. Each antigen was eluted off the column with a increasing salt gradient. Fractions were collected as the column was run and another SDS-PAGE gel was run to determine which fractions from the column to pool. The pooled fractions were dialyzed against 10 mM Tris pH 8.0. The proteins were then vialled after filtration through a 0.22 micron filter and the antigens were frozen until needed for immunization.

Four hundred micrograms of each prostate antigen was combined with 100 micrograms of muramyl dipeptide (MDP). Every four weeks rabbits were boosted with 100 micrograms mixed with an equal volume of Incomplete Freund's Adjuvant (IFA). Seven days following each boost, the animal was bled. Sera was generated by incubating the blood at 4°C for 12-4 hours followed by centrifugation.

Ninety-six well plates were coated with antigen by incubating with 50 microliters (typically 1 microgram) of recombinant protein at 4 °C for 20 hours. 250 microliters of BSA blocking buffer was added to the wells and incubated at room temperature for 2 hours. Plates were washed 6 times with PBS/0.01% Tween. Rabbit sera was diluted in PBS. Fifty microliters of diluted sera was added to each well and incubated at room temperature for 30 min. Plates were washed as described above before 50 microliters of goat anti-rabbit horse radish peroxidase (HRP) at a 1:10000 dilution was added and incubated at room temperature for 30 min. Plates were again

washed as described above and 100 microliters of TMB microwell peroxidase substrate was added to each well. Following a 15 min incubation in the dark at room temperature, the colorimetric reaction was stopped with 100 microliters of 1N H₂SO₄ and read immediately at 450 nm. All polyclonal antibodies showed immunoreactivity to the appropriate antigen.

b) Preparation and Characterization of Antibodies against P501S

A murine monoclonal antibody directed against the carboxy-terminus of the prostate-specific antigen P501S was prepared as follows.

A truncated fragment of P501S (amino acids 355-526 of SEQ ID NO: 113) was generated and cloned into the pET28b vector (Novagen) and expressed in *E. coli* as a thioredoxin fusion protein with a histidine tag. The trx-P501S fusion protein was purified by nickel chromatography, digested with thrombin to remove the trx fragment and further purified by an acid precipitation procedure followed by reverse phase HPLC.

Mice were immunized with truncated P501S protein. Serum bleeds from mice that potentially contained anti-P501S polyclonal sera were tested for P501S-specific reactivity using ELISA assays with purified P501S and trx-P501S proteins. Serum bleeds that appeared to react specifically with P501S were then screened for P501S reactivity by Western analysis. Mice that contained a P501S-specific antibody component were sacrificed and spleen cells were used to generate anti-P501S antibody producing hybridomas using standard techniques. Hybridoma supernatants were tested for P501S-specific reactivity initially by ELISA, and subsequently by FACS analysis of reactivity with P501S transduced cells. Based on these results, a monoclonal hybridoma referred to as 10E3 was chosen for further subcloning. A number of subclones were generated, tested for specific reactivity to P501S using ELISA and typed for IgG isotype. The results of this analysis are shown below in Table V. Of the 16 subclones tested, the monoclonal antibody 10E3-G4-D3 was selected for further study.

Table V

Isotype analysis of murine anti-P501S monoclonal antibodies

Hybridoma clone	Isotype	Estimated [Ig] in supernatant ($\mu\text{g/ml}$)
4D11	IgG1	14.6
1G1	IgG1	0.6
4F6	IgG1	72
4H5	IgG1	13.8
4H5-E12	IgG1	10.7
4H5-EH2	IgG1	9.2
4H5-H2-A10	IgG1	10
4H5-H2-A3	IgG1	12.8
4H5-H2-A10-G6	IgG1	13.6
4H5-H2-B11	IgG1	12.3
10E3	IgG2a	3.4
10E3-D4	IgG2a	3.8
10E3-D4-G3	IgG2a	9.5
10E3-D4-G6	IgG2a	10.4
10E3-E7	IgG2a	6.5
8H12	IgG2a	0.6

5 The specificity of 10E3-G4-D3 for P501S was examined by FACS analysis. Specifically, cells were fixed (2% formaldehyde, 10 minutes), permeabilized (0.1% saponin, 10 minutes) and stained with 10E3-G4-D3 at 0.5 – 1 $\mu\text{g/ml}$, followed by incubation with a secondary, FITC-conjugated goat anti-mouse Ig antibody (Pharmingen, San Diego, CA). Cells were then analyzed for FITC fluorescence using

10 an Excalibur fluorescence activated cell sorter. For FACS analysis of transduced cells, B-LCL were retrovirally transduced with P501S. For analysis of infected cells, B-LCL were infected with a vaccinia vector that expresses P501S. To demonstrate specificity in these assays, B-LCL transduced with a different antigen (P703P) and uninfected B-LCL vectors were utilized. 10E3-G4-D3 was shown to bind with P501S-transduced B-

15 LCL and also with P501S-infected B-LCL, but not with either uninfected cells or P703P-transduced cells.

To determine whether the epitope recognized by 10E3-G4-D3 was found on the surface or in an intracellular compartment of cells, B-LCL were transduced with P501S or HLA-B8 as a control antigen and either fixed and permeabilized as described

above or directly stained with 10E3-G4-D3 and analyzed as above. Specific recognition of P501S by 10E3-G4-D3 was found to require permeabilization, suggesting that the epitope recognized by this antibody is intracellular.

The reactivity of 10E3-G4-D3 with the three prostate tumor cell lines Lncap, PC-3 and DU-145, which are known to express high, medium and very low levels of P501S, respectively, was examined by permeabilizing the cells and treating them as described above. Higher reactivity of 10E3-G4-D3 was seen with Lncap than with PC-3, which in turn showed higher reactivity than DU-145. These results are in agreement with the real time PCR and demonstrate that the antibody specifically recognizes P501S in these tumor cell lines and that the epitope recognized in prostate tumor cell lines is also intracellular.

Specificity of 10E3-G4-D3 for P501S was also demonstrated by Western blot analysis. Lysates from the prostate tumor cell lines Lncap, DU-145 and PC-3, from P501S-transiently transfected HEK293 cells, and from non-transfected HEK293 cells were generated. Western blot analysis of these lysates with 10E3-G4-D3 revealed a 46 kDa immunoreactive band in Lncap, PC-3 and P501S-transfected HEK cells, but not in DU-145 cells or non-transfected HEK293 cells. P501S mRNA expression is consistent with these results since semi-quantitative PCR analysis revealed that P501S mRNA is expressed in Lncap, to a lesser but detectable level in PC-3 and not at all in DU-145 cells. Bacterially expressed and purified recombinant P501S (referred to as P501SStr2) was recognized by 10E3-G4-D3 (24 kDa), as was full-length P501S that was transiently expressed in HEK293 cells using either the expression vector VR1012 or pCEP4. Although the predicted molecular weight of P501S is 60.5 kDa, both transfected and "native" P501S run at a slightly lower mobility due to its hydrophobic nature.

Immunohistochemical analysis was performed on prostate tumor and a panel of normal tissue sections (prostate, adrenal, breast, cervix, colon, duodenum, gall bladder, ileum, kidney, ovary, pancreas, parotid gland, skeletal muscle, spleen and testis). Tissue samples were fixed in formalin solution for 24 hours and embedded in paraffin before being sliced into 10 micron sections. Tissue sections were permeabilized and incubated with 10E3-G4-D3 antibody for 1 hr. HRP-labeled anti-

mouse followed by incubation with DAB chromogen was used to visualize P501S immunoreactivity. P501S was found to be highly expressed in both normal prostate and prostate tumor tissue but was not detected in any of the other tissues tested.

To identify the epitope recognized by 10E3-G4-D3, an epitope mapping approach was pursued. A series of 13 overlapping 20-21 mers (5 amino acid overlap; SEQ ID NO: 489-501) was synthesized that spanned the fragment of P501S used to generate 10E3-G4-D3. Flat bottom 96 well microtiter plates were coated with either the peptides or the P501S fragment used to immunize mice, at 1 microgram/ml for 2 hours at 37 °C. Wells were then aspirated and blocked with phosphate buffered saline containing 1% (w/v) BSA for 2 hours at room temperature, and subsequently washed in PBS containing 0.1% Tween 20 (PBST). Purified antibody 10E3-G4-D3 was added at 2 fold dilutions (1000 ng – 16 ng) in PBST and incubated for 30 minutes at room temperature. This was followed by washing 6 times with PBST and subsequently incubating with HRP-conjugated donkey anti-mouse IgG (H+L) Affinipure F(ab') fragment (Jackson ImmunoResearch, West Grove, PA) at 1:20000 for 30 minutes. Plates were then washed and incubated for 15 minutes in tetramethyl benzidine. Reactions were stopped by the addition of 1N sulfuric acid and plates were read at 450 nm using an ELISA plate reader. As shown in Fig. 8, reactivity was seen with the peptide of SEQ ID NO: 496 (corresponding to amino acids 439-459 of P501S) and with the P501S fragment but not with the remaining peptides, demonstrating that the epitope recognized by 10E3-G4-D3 is localized to amino acids 439-459 of SEQ ID NO: 113.

In order to further evaluate the tissue specificity of P501S, multi-array immunohistochemical analysis was performed on approximately 4700 different human tissues encompassing all the major normal organs as well as neoplasias derived from these tissues. Sixty-five of these human tissue samples were of prostate origin. Tissue sections 0.6 mm in diameter were formalin-fixed and paraffin embedded. Samples were pretreated with HIER using 10 mM citrate buffer pH 6.0 and boiling for 10 min. Sections were stained with 10E3-G4-D3 and P501S immunoreactivity was visualized with HRP. All the 65 prostate tissues samples (5 normal, 55 untreated prostate tumors,

5 hormone refractory prostate tumors) were positive, showing distinct perinuclear staining. All other tissues examined were negative for P501S expression.

c) Preparation and Characterization of Antibodies against P503S

5 A fragment of P503S (amino acids 113-241 of SEQ ID NO: 114) was expressed and purified from bacteria essentially as described above for P501S and used to immunize both rabbits and mice. Mouse monoclonal antibodies were isolated using standard hybridoma technology as described above. Rabbit monoclonal antibodies were isolated using Selected Lymphocyte Antibody Method (SLAM) technology at
 10 Immugenics Pharmaceuticals (Vancouver, BC, Canada). Table VI, below, lists the monoclonal antibodies that were developed against P503S.

Table VI

Antibody	Species
20D4	Rabbit
JA1	Rabbit
1A4	Mouse
1C3	Mouse
1C9	Mouse
1D12	Mouse
2A11	Mouse
2H9	Mouse
4H7	Mouse
8A8	Mouse
8D10	Mouse
9C12	Mouse
6D12	Mouse

15

The DNA sequences encoding the complementarity determining regions (CDRs) for the rabbit monoclonal antibodies 20D4 and JA1 were determined and are provided in SEQ ID NO: 502 and 503, respectively.

In order to better define the epitope binding region of each of the antibodies, a series of overlapping peptides were generated that span amino acids 109-213 of SEQ ID NO: 114. These peptides were used to epitope map the anti-P503S monoclonal antibodies by ELISA as follows. The recombinant fragment of P503S that was employed as the immunogen was used as a positive control. Ninety-six well microtiter plates were coated with either peptide or recombinant antigen at 20 ng/well overnight at 4 °C. Plates were aspirated and blocked with phosphate buffered saline containing 1% (w/v) BSA for 2 hours at room temperature then washed in PBS containing 0.1% Tween 20 (PBST). Purified rabbit monoclonal antibodies diluted in PBST were added to the wells and incubated for 30 min at room temperature. This was followed by washing 6 times with PBST and incubation with Protein-A HRP conjugate at a 1:2000 dilution for a further 30 min. Plates were washed six times in PBST and incubated with tetramethylbenzidine (TMB) substrate for a further 15 min. The reaction was stopped by the addition of 1N sulfuric acid and plates were read at 450 nm using at ELISA plate reader. ELISA with the mouse monoclonal antibodies was performed with supernatants from tissue culture run neat in the assay.

All of the antibodies bound to the recombinant P503S fragment, with the exception of the negative control SP2 supernatant. 20D4, JA1 and ID12 bound strictly to peptide #2101 (SEQ ID NO: 504), which corresponds to amino acids 151-169 of SEQ ID NO: 114. 1C3 bound to peptide #2102 (SEQ ID NO: 505), which corresponds to amino acids 165-184 of SEQ ID NO: 114. 9C12 bound to peptide #2099 (SEQ ID NO: 522), which corresponds to amino acids 120-139 of SEQ ID NO: 114. The other antibodies bind to regions that were not examined in these studies.

Subsequent to epitope mapping, the antibodies were tested by FACS analysis on a cell line that stably expressed P503S to confirm that the antibodies bind to cell surface epitopes. Cells stably transfected with a control plasmid were employed as a negative control. Cells were stained live with no fixative. 0.5 ug of anti-P503S monoclonal antibody was added and cells were incubated on ice for 30 min before being washed twice and incubated with a FITC-labelled goat anti-rabbit or mouse secondary antibody for 20 min. After being washed twice, cells were analyzed with an Excalibur

fluorescent activated cell sorter. The monoclonal antibodies 1C3, 1D12, 9C12, 20D4 and JA1, but not 8D3, were found to bind to a cell surface epitope of P503S.

In order to determine which tissues express P503S, immunohistochemical analysis was performed, essentially as described above, on a panel of normal tissues (prostate, adrenal, breast, cervix, colon, duodenum, gall bladder, ileum, kidney, ovary, pancreas, parotid gland, skeletal muscle, spleen and testis). HRP-labeled anti-mouse or anti-rabbit antibody followed by incubation with TMB was used to visualize P503S immunoreactivity. P503S was found to be highly expressed in prostate tissue, with lower levels of expression being observed in cervix, colon, ileum and kidney, and no expression being observed in adrenal, breast, duodenum, gall bladder, ovary, pancreas, parotid gland, skeletal muscle, spleen and testis.

Western blot analysis was used to characterize anti-P503S monoclonal antibody specificity. SDS-PAGE was performed on recombinant (rec) P503S expressed in and purified from bacteria and on lysates from HEK293 cells transfected with full length P503S. Protein was transferred to nitrocellulose and then Western blotted with each of the anti-P503S monoclonal antibodies (20D4, JA1, 1D12, 6D12 and 9C12) at an antibody concentration of 1 ug/ml. Protein was detected using horse radish peroxidase (HRP) conjugated to either a goat anti-mouse monoclonal antibody or to protein A-sepharose. The monoclonal antibody 20D4 detected the appropriate molecular weight 14 kDa recombinant P503S (amino acids 113-241) and the 23.5 kDa species in the HEK293 cell lysates transfected with full length P503S. Other anti-P503S monoclonal antibodies displayed similar specificity by Western blot.

d) Preparation and Characterization of Antibodies against P703P

Rabbits were immunized with either a truncated (P703Ptr1; SEQ ID NO: 172) or full-length mature form (P703Pfl; SEQ ID NO: 523) of recombinant P703P protein was expressed in and purified from bacteria as described above. Affinity purified polyclonal antibody was generated using immunogen P703Pfl or P703Ptr1 attached to a solid support. Rabbit monoclonal antibodies were isolated using SLAM

technology at Immgenics Pharmaceuticals. Table VII below lists both the polyclonal and monoclonal antibodies that were generated against P703P.

Table VII

Antibody	Immunogen	Species/type
Aff. Purif. P703P (truncated); #2594	P703Ptrl	Rabbit polyclonal
Aff. Purif. P703P (full length); #9245	P703Pfl	Rabbit polyclonal
2D4	P703Ptrl	Rabbit monoclonal
8H2	P703Ptrl	Rabbit monoclonal
7H8	P703Ptrl	Rabbit monoclonal

The DNA sequences encoding the complementarity determining regions (CDRs) for the rabbit monoclonal antibodies 8H2, 7H8 and 2D4 were determined and are provided in SEQ ID NO: 506-508, respectively.

Epitope mapping studies were performed as described above. Monoclonal antibodies 2D4 and 7H8 were found to specifically bind to the peptides of SEQ ID NO: 509 (corresponding to amino acids 145-159 of SEQ ID NO: 172) and SEQ ID NO: 510 (corresponding to amino acids 11-25 of SEQ ID NO: 172), respectively. The polyclonal antibody 2594 was found to bind to the peptides of SEQ ID NO: 511-514, with the polyclonal antibody 9427 binding to the peptides of SEQ ID NO: 515-517.

The specificity of the anti-P703P antibodies was determined by Western blot analysis as follows. SDS-PAGE was performed on (1) bacterially expressed recombinant antigen; (2) lysates of HEK293 cells and Ltk^{-/-} cells either untransfected or transfected with a plasmid expressing full length P703P; and (3) supernatant isolated from these cell cultures. Protein was transferred to nitrocellulose and then Western blotted using the anti-P703P polyclonal antibody #2594 at an antibody concentration of 1 ug/ml. Protein was detected using horse radish peroxidase (HRP) conjugated to an anti-rabbit antibody. A 35 kDa immunoreactive band could be observed with recombinant P703P. Recombinant P703P runs at a slightly higher molecular weight since it is epitope tagged. In lysates and supernatants from cells transfected with full length P703P, a 30 kDa band corresponding to P703P was observed. To assure

specificity, lysates from HEK293 cells stably transfected with a control plasmid were also tested and were negative for P703P expression. Other anti-P703P antibodies showed similar results.

Immunohistochemical studies were performed as described above, using anti-P703P monoclonal antibody. P703P was found to be expressed at high levels in normal prostate and prostate tumor tissue but was not detectable in all other tissues tested (breast tumor, lung tumor and normal kidney).

e) Preparation and Characterization of Antibodies against P504S

Full-length P504S (SEQ ID NO: 108) was expressed and purified from bacteria essentially as described above for P501S and employed to raise rabbit monoclonal antibodies using Selected Lymphocyte Antibody Method (SLAM) technology at Immgenics Pharmaceuticals (Vancouver, BC, Canada). The anti-P504S monoclonal antibody 13H4 was shown by Western blot to bind to both expressed recombinant P504S and to native P504S in tumor cells.

Immunohistochemical studies using 13H4 to assess P504S expression in various prostate tissues were performed as described above. A total of 104 cases, including 65 cases of radical prostatectomies with prostate cancer (PC), 26 cases of prostate biopsies and 13 cases of benign prostate hyperplasia (BPH), were stained with the anti-P504S monoclonal antibody 13H4. P504S showed strongly cytoplasmic granular staining in 64/65 (98.5%) of PCs in prostatectomies and 26/26 (100%) of PCs in prostatic biopsies. P504S was stained strongly and diffusely in carcinomas (4+ in 91.2% of cases of PC; 3+ in 5.5%; 2+ in 2.2% and 1+ in 1.1%) and high grade prostatic intraepithelial neoplasia (4+ in all cases). The expression of P504S did not vary with Gleason score. Only 17/91 (18.7%) of cases of NP/BPH around PC and 2/13 (15.4%) of BPH cases were focally (1+, no 2+ to 4+ in all cases) and weakly positive for P504S in large glands. Expression of P504S was not found in small atrophic glands, postatrophic hyperplasia, basal cell hyperplasia and transitional cell metaplasia in either biopsies or prostatectomies. P504S was thus found to be over-expressed in all Gleason scores of prostate cancer (98.5 to 100% of sensitivity) and exhibited only focal positivities in

large normal glands in 19/104 of cases (82.3% of specificity). These findings indicate that P504S may be usefully employed for the diagnosis of prostate cancer.

EXAMPLE 19

CHARACTERIZATION OF CELL SURFACE EXPRESSION AND CHROMOSOME LOCALIZATION OF THE PROSTATE-SPECIFIC ANTIGEN P501S

This example describes studies demonstrating that the prostate-specific antigen P501S is expressed on the surface of cells, together with studies to determine the probable chromosomal location of P501S.

The protein P501S (SEQ ID NO: 113) is predicted to have 11 transmembrane domains. Based on the discovery that the epitope recognized by the anti-P501S monoclonal antibody 10E3-G4-D3 (described above in Example 17) is intracellular, it was predicted that following transmembrane determinants would allow the prediction of extracellular domains of P501S. Fig. 9 is a schematic representation of the P501S protein showing the predicted location of the transmembrane domains and the intracellular epitope described in Example 17. Underlined sequence represents the predicted transmembrane domains, bold sequence represents the predicted extracellular domains, and italicized sequence represents the predicted intracellular domains. Sequence that is both bold and underlined represents sequence employed to generate polyclonal rabbit serum. The location of the transmembrane domains was predicted using HHMTOP as described by Tusnady and Simon (Principles Governing Amino Acid Composition of Integral Membrane Proteins: Applications to Topology Prediction, *J. Mol. Biol.* 283:489-506, 1998).

Based on Fig. 9, the P501S domain flanked by the transmembrane domains corresponding to amino acids 274-295 and 323-342 is predicted to be extracellular. The peptide of SEQ ID NO: 518 corresponds to amino acids 306-320 of P501S and lies in the predicted extracellular domain. The peptide of SEQ ID NO: 519, which is identical to the peptide of SEQ ID NO: 518 with the exception of the substitution of the histidine with an asparagine, was synthesized as described above. A

Cys-Gly was added to the C-terminus of the peptide to facilitate conjugation to the carrier protein. Cleavage of the peptide from the solid support was carried out using the following cleavage mixture: trifluoroacetic acid:ethanediol:thioanisol:water:phenol (40:1:2:2:3). After cleaving for two hours, the peptide was precipitated in cold ether.

- 5 The peptide pellet was then dissolved in 10% v/v acetic acid and lyophilized prior to purification by C18 reverse phase hplc. A gradient of 5-60% acetonitrile (containing 0.05% TFA) in water (containing 0.05% TFA) was used to elute the peptide. The purity of the peptide was verified by hplc and mass spectrometry, and was determined to be >95%. The purified peptide was used to generate rabbit polyclonal antisera as described
10 above.

- Surface expression of P501S was examined by FACS analysis. Cells were stained with the polyclonal anti-P501S peptide serum at 10 µg/ml, washed, incubated with a secondary FITC-conjugated goat anti-rabbit Ig antibody (ICN), washed and analyzed for FITC fluorescence using an Excalibur fluorescence activated cell
15 sorter. For FACS analysis of transduced cells, B-LCL were retrovirally transduced with P501S. To demonstrate specificity in these assays, B-LCL transduced with an irrelevant antigen (P703P) or nontransduced were stained in parallel. For FACS analysis of prostate tumor cell lines, Lncap, PC-3 and DU-145 were utilized. Prostate tumor cell lines were dissociated from tissue culture plates using cell dissociation medium and
20 stained as above. All samples were treated with propidium iodide (PI) prior to FACS analysis, and data was obtained from PI-excluding (i.e., intact and non-permeabilized) cells. The rabbit polyclonal serum generated against the peptide of SEQ ID NO: 519 was shown to specifically recognize the surface of cells transduced to express P501S, demonstrating that the epitope recognized by the polyclonal serum is extracellular.

- 25 To determine biochemically if P501S is expressed on the cell surface, peripheral membranes from Lncap cells were isolated and subjected to Western blot analysis. Specifically, Lncap cells were lysed using a dounce homogenizer in 5 ml of homogenization buffer (250 mM sucrose, 10 mM HEPES, 1mM EDTA, pH 8.0, 1 complete protease inhibitor tablet (Boehringer Mannheim)). Lysate samples were spun
30 at 1000 g for 5 min at 4 °C. The supernatant was then spun at 8000g for 10 min at 4 °C.

Supernatant from the 8000g spin was recovered and subjected to a 100,000g spin for 30 min at 4 °C to recover peripheral membrane. Samples were then separated by SDS-PAGE and Western blotted with the mouse monoclonal antibody 10E3-G4-D3 (described above in Example 17) using conditions described above. Recombinant purified P501S, as well as HEK293 cells transfected with and over-expressing P501S were included as positive controls for P501S detection. LCL cell lysate was included as a negative control. P501S could be detected in Lncap total cell lysate, the 8000g (internal membrane) fraction and also in the 100,000g (plasma membrane) fraction. These results indicate that P501S is expressed at, and localizes to, the peripheral membrane.

To demonstrate that the rabbit polyclonal antiserum generated to the peptide of SEQ ID NO: 519 specifically recognizes this peptide as well as the corresponding native peptide of SEQ ID NO: 518, ELISA analyses were performed. For these analyses, flat-bottomed 96 well microtiter plates were coated with either the peptide of SEQ ID NO: 519, the longer peptide of SEQ ID NO: 520 that spans the entire predicted extracellular domain, the peptide of SEQ ID NO: 521 which represents the epitope recognized by the P501S-specific antibody 10E3-G4-D3, or a P501S fragment (corresponding to amino acids 355-526 of SEQ ID NO: 113) that does not include the immunizing peptide sequence, at 1 µg/ml for 2 hours at 37 °C. Wells were aspirated, blocked with phosphate buffered saline containing 1% (w/v) BSA for 2 hours at room temperature and subsequently washed in PBS containing 0.1% Tween 20 (PBST). Purified anti-P501S polyclonal rabbit serum was added at 2 fold dilutions (1000 ng - 125 ng) in PBST and incubated for 30 min at room temperature. This was followed by washing 6 times with PBST and incubating with HRP-conjugated goat anti-rabbit IgG (H+L) Affinipure F(ab') fragment at 1:20000 for 30 min. Plates were then washed and incubated for 15 min in tetramethyl benzidine. Reactions were stopped by the addition of 1N sulfuric acid and plates were read at 450 nm using an ELISA plate reader. As shown in Fig. 11, the anti-P501S polyclonal rabbit serum specifically recognized the peptide of SEQ ID NO: 519 used in the immunization as well as the longer peptide of

SEQ ID NO: 520, but did not recognize the irrelevant P501S-derived peptides and fragments.

In further studies, rabbits were immunized with peptides derived from the P501S sequence and predicted to be either extracellular or intracellular, as shown in Fig. 9. Polyclonal rabbit sera were isolated and polyclonal antibodies in the serum were purified, as described above. To determine specific reactivity with P501S, FACS analysis was employed, utilizing either B-LCL transduced with P501S or the irrelevant antigen P703P, of B-LCL infected with vaccinia virus-expressing P501S. For surface expression, dead and non-intact cells were excluded from the analysis as described above. For intracellular staining, cells were fixed and permeabilized as described above. Rabbit polyclonal serum generated against the peptide of SEQ ID NO: 548, which corresponds to amino acids 181-198 of P501S, was found to recognize a surface epitope of P501S. Rabbit polyclonal serum generated against the peptide SEQ ID NO: 551, which corresponds to amino acids 543-553 of P501S, was found to recognize an epitope that was either potentially extracellular or intracellular since in different experiments intact or permeabilized cells were recognized by the polyclonal sera. Based on similar deductive reasoning, the sequences of SEQ ID NO: 541-547, 549 and 550, which correspond to amino acids 109-122, 539-553, 509-520, 37-54, 342-359, 295-323, 217-274, 143-160 and 75-88, respectively, of P501S, can be considered to be potential surface epitopes of P501S recognized by antibodies.

In further studies, mouse monoclonal antibodies were raised against amino acids 296 to 322 to P501S, which are predicted to be in an extracellular domain. A/J mice were immunized with P501S/adenovirus, followed by subsequent boosts with an *E. coli* recombinant protein, referred to as P501N, that contains amino acids 296 to 322 of P501S, and with peptide 296-322 (SEQ ID NO: 898) coupled with KLH. The mice were subsequently used for splenic B cell fusions to generate anti-peptide hybridomas. The resulting 3 clones, referred to as 4F4 (IgG1,kappa), 4G5 (IgG2a,kappa) and 9B9 (IgG1,kappa), were grown for antibody production. The 4G5 mAb was purified by passing the supernatant over a Protein A-sepharose column,

followed by antibody elution using 0.2M glycine, pH 2.3. Purified antibody was neutralized by the addition of 1M Tris, pH 8, and buffer exchanged into PBS.

For ELISA analysis, 96 well plates were coated with P501S peptide 296-322 (referred to as P501-long), an irrelevant P775 peptide, P501S-N, P501TR2, P501S-long-KLH, P501S peptide 306-319 (referred to as P501-short)-KLH, or the irrelevant peptide 2073-KLH, all at a concentration of 2 ug/ml and allowed to incubate for 60 minutes at 37 °C. After coating, plates were washed 5X with PBS + 0.1% Tween and then blocked with PBS, 0.5% BSA, 0.4% Tween20 for 2 hours at room temperature. Following the addition of supernatants or purified mAb, the plates were incubated for 60 minutes at room temperature. Plates were washed as above and donkey anti-mouse IgHRP-linked secondary antibody was added and incubated for 30 minutes at room temperature, followed by a final washing as above. TMB peroxidase substrate was added and incubated 15 minutes at room temperature in the dark. The reaction was stopped by the addition of 1N H₂SO₄ and the OD was read at 450 nM. All three hybrid clones secreted mAb that recognized peptide 296-322 and the recombinant protein P501N.

For FACS analysis, HEK293 cells were transiently transfected with a P501S/VR1012 expression constructs using Fugene 6 reagent. After 2 days of culture, cells were harvested and washed, then incubated with purified 4G5 mAb for 30 minutes on ice. After several washes in PBS, 0.5% BSA, 0.01% azide, goat anti-mouse Ig-FITC was added to the cells and incubated for 30 minutes on ice. Cells were washed and resuspended in wash buffer including 1% propidium iodide and subjected to FACS analysis. The FACS analysis confirmed that amino acids 296-322 of P501S are in an extracellular domain and are cell surface expressed.

The chromosomal location of P501S was determined using the GeneBridge 4 Radiation Hybrid panel (Research Genetics). The PCR primers of SEQ ID NO: 528 and 529 were employed in PCR with DNA pools from the hybrid panel according to the manufacturer's directions. After 38 cycles of amplification, the reaction products were separated on a 1.2% agarose gel, and the results were analyzed through the Whitehead Institute/MIT Center for Genome Research web server

(<http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl>) to determine the probable chromosomal location. Using this approach, P501S was mapped to the long arm of chromosome 1 at WI-9641 between q32 and q42. This region of chromosome 1 has been linked to prostate cancer susceptibility in hereditary prostate cancer (Smith *et al.* *Science* 274:1371-1374, 1996 and Berthon *et al. Am. J. Hum. Genet.* 62:1416-1424, 1998). These results suggest that P501S may play a role in prostate cancer malignancy.

EXAMPLE 20

REGULATION OF EXPRESSION OF THE PROSTATE-SPECIFIC ANTIGEN P501S

10

Steroid (androgen) hormone modulation is a common treatment modality in prostate cancer. The expression of a number of prostate tissue-specific antigens have previously been demonstrated to respond to androgen. The responsiveness of the prostate-specific antigen P501S to androgen treatment was examined in a tissue culture system as follows.

15

Cells from the prostate tumor cell line LNCaP were plated at 1.5×10^6 cells/T75 flask (for RNA isolation) or 3×10^5 cells/well of a 6-well plate (for FACS analysis) and grown overnight in RPMI 1640 media containing 10% charcoal-stripped fetal calf serum (BRL Life Technologies, Gaithersburg, MD). Cell culture was continued for an additional 72 hours in RPMI 1640 media containing 10% charcoal-stripped fetal calf serum, with 1 nM of the synthetic androgen Methyltrienolone (R1881; New England Nuclear) added at various time points. Cells were then harvested for RNA isolation and FACS analysis at 0, 1, 2, 4, 8, 16, 24, 28 and 72-hours post androgen addition. FACS analysis was performed using the anti-P501S antibody 10E3-G4-D3 and permeabilized cells.

25

For Northern analysis, 5-10 micrograms of total RNA was run on a formaldehyde denaturing gel, transferred to Hybond-N nylon membrane (Amersham Pharmacia Biotech, Piscataway, NJ), cross-linked and stained with methylene blue. The filter was then prehybridized with Church's Buffer (250 mM Na_2HPO_4 , 70 mM H_3PO_4 , 1 mM EDTA, 1% SDS, 1% BSA in pH 7.2) at 65 °C for 1 hour. P501S DNA was

30

labeled with ^{32}P using High Prime random-primed DNA labeling kit (Boehringer Mannheim). Unincorporated label was removed using MicroSpin S300-HR columns (Amersham Pharmacia Biotech). The RNA filter was then hybridized with fresh Church's Buffer containing labeled cDNA overnight, washed with 1X SCP (0.1 M NaCl, 0.03 M $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 0.001 M Na_2EDTA), 1% sarkosyl (n-lauroylsarcosine) and exposed to X-ray film.

Using both FACS and Northern analysis, P5018 message and protein levels were found to increase in response to androgen treatment.

10

EXAMPLE 21

PREPARATION OF FUSION PROTEINS OF PROSTATE-SPECIFIC ANTIGENS

The example describes the preparation of a fusion protein of the prostate-specific antigen P703P and a truncated form of the known prostate antigen PSA. The truncated form of PSA has a 21 amino acid deletion around the active serine site. The expression construct for the fusion protein also has a restriction site at 3' end, immediately prior to the termination codon, to aid in adding cDNA for additional antigens.

The full-length cDNA for PSA was obtained by RT-PCR from a pool of RNA from human prostate tumor tissues using the primers of SEQ ID NO: 607 and 608, and cloned in the vector pCR-Blunt II-TOPO. The resulting cDNA was employed as a template to make two different fragments of PSA by PCR with two sets of primers (SEQ ID NO: 609 and 610; and SEQ ID NO: 611 and 612). The PCR products having the expected size were used as templates to make truncated forms of PSA by PCR with the primers of SEQ ID NO: 611 and 613, which generated PSA (delta 208-218 in amino acids). The cDNA for the mature form of P703P with a 6X histidine tag at the 5' end, was prepared by PCR with P703P and the primers of SEQ ID NO: 614 and 615. The cDNA for the fusion of P703P with the truncated form of PSA (referred to as FOPP) was then obtained by PCR using the modified P703P cDNA and the truncated form of PSA cDNA as templates and the primers of SEQ ID NO: 614 and 615. The FOPP

cDNA was cloned into the NdeI site and XhoI site of the expression vector pCRX1, and confirmed by DNA sequencing. The determined cDNA sequence for the fusion construct FOPP is provided in SEQ ID NO: 616, with the amino acid sequence being provided in SEQ ID NO: 617.

5 The fusion FOPP was expressed as a single recombinant protein in *E. coli* as follows. The expression plasmid pCRX1FOPP was transformed into the *E. coli* strain BL21-CodonPlus RIL. The transformant was shown to express FOPP protein upon induction with 1 mM IPTG. The culture of the corresponding expression clone was inoculated into 25 ml LB broth containing 50 ug/ml kanamycin and 34 ug/ml
10 chloramphenicol, grown at 37 °C to OD600 of about 1, and stored at 4 °C overnight. The culture was diluted into 1 liter of TB LB containing 50 ug/ml kanamycin and 34 ug/ml chloramphenicol, and grown at 37 °C to OD600 of 0.4. IPTG was added to a final concentration of 1 mM, and the culture was incubated at 30 °C for 3 hours. The cells were pelleted by centrifugation at 5,000 RPM for 8 min. To purify the protein, the
15 cell pellet was suspended in 25 ml of 10 mM Tris-Cl pH 8.0, 2mM PMSF, complete protease inhibitor and 15 ug lysozyme. The cells were lysed at 4 °C for 30 minutes, sonicated several times and the lysate centrifuged for 30 minutes at 10,000 x g. The precipitate, which contained the inclusion body, was washed twice with 10 mM Tris-Cl pH 8.0 and 1% CHAPS. The inclusion body was dissolved in 40 ml of 10 mM Tris-Cl
20 pH 8.0, 100 mM sodium phosphate and 8 M urea. The solution was bound to 8 ml Ni-NTA (Qiagen) for one hour at room temperature. The mixture was poured into a 25 ml column and washed with 50 ml of 10 mM Tris-Cl pH 6.3, 100 mM sodium phosphate, 0.5% DOC and 8M urea. The bound protein was eluted with 350 mM imidazole, 10 mM Tris-Cl pH 8.0, 100 mM sodium phosphate and 8 M urea. The fractions containing
25 FOPP proteins were combined and dialyzed extensively against 10 mM Tris-Cl pH 4.6, aliquoted and stored at - 70 °C.

EXAMPLE 22

REAL-TIME PCR CHARACTERIZATION OF THE PROSTATE-SPECIFIC ANTIGEN P501S IN
PERIPHERAL BLOOD OF PROSTATE CANCER PATIENTS

5 Circulating epithelial cells were isolated from fresh blood of normal individuals and metastatic prostate cancer patients, mRNA isolated and cDNA prepared using real-time PCR procedures. Real-time PCR was performed with the TaqmanTM procedure using both gene specific primers and probes to determine the levels of gene expression.

10 Epithelial cells were enriched from blood samples using an immunomagnetic bead separation method (Dynal A.S., Oslo, Norway). Isolated cells were lysed and the magnetic beads removed. The lysate was then processed for poly A+ mRNA isolation using magnetic beads coated with Oligo(dT)25. After washing the beads in buffer, bead/poly A+ RNA samples were suspended in 10 mM Tris HCl pH 8.0
15 and subjected to reversed transcription. The resulting cDNA was subjected to real-time PCR using gene specific primers. Beta-actin content was also determined and used for normalization. Samples with P501S copies greater than the mean of the normal samples + 3 standard deviations were considered positive. Real time PCR on blood samples was performed using the TaqmanTM procedure but extending to 50 cycles using
20 forward and reverse primers and probes specific for P501S. Of the eight samples tested, 6 were positive for P501S and β -actin signal. The remaining 2 samples had no detectable β -actin or P501S. No P501S signal was observed in the four normal blood samples tested.

EXAMPLE 23

EXPRESSION OF THE PROSTATE-SPECIFIC ANTIGENS P703P AND P501S IN
SCID MOUSE-PASSAGED PROSTATE TUMORS

25 When considering the effectiveness of antigens in the treatment of
30 prostate cancer, the continued presence of the antigens in tumors during androgen

ablation therapy is important. The presence of the prostate-specific antigens P703P and P501S in prostate tumor samples grown in SCID mice in the presence of testosterone was evaluated as follows.

Two prostate tumors that had metastasized to the bone were removed
5 from patients, implanted into SCID mice and grown in the presence of testosterone. Tumors were evaluated for mRNA expression of P703P, P501S and PSA using quantitative real time PCR with the SYBR green assay method. Expression of P703P and P501S in a prostate tumor was used as a positive control and the absence in normal intestine and normal heart as negative controls. In both cases, the specific mRNA was
10 present in late passage tumors. Since the bone metastases were grown in the presence of testosterone, this implies that the presence of these genes would not be lost during androgen ablation therapy.

EXAMPLE 24

15 ANTI-P503S MONOCLONAL ANTIBODY INHIBITS TUMOR GROWTH *IN VIVO*

The ability of the anti-P503S monoclonal antibody 20D4 to suppress tumor formation in mice was examined as follows.

Ten SCID mice were injected subcutaneously with HEK293 cells that expressed P503S. Five mice received 150 micrograms of 20D4 intravenously at day 0
20 (time of tumor cell injection), day 5 and day 9. Tumor size was measured for 50 days. Of the five animals that received no 20D4, three formed detectable tumors after about 2 weeks which continued to enlarge throughout the study. In contrast, none of the five mice that received 20D4 formed tumors. These results demonstrate that the anti-P503S Mab 20D4 displays potent anti-tumor activity *in vivo*.

25

EXAMPLE 25

CHARACTERIZATION OF A T CELL RECEPTOR CLONE FROM A P501S-SPECIFIC T CELL CLONE

30 T cells have a limited lifespan. However, cloning of T cell receptor (TCR) chains and subsequent transfer essentially enables infinite propagation of the T

cell specificity. Cloning of tumor-antigen TCR chains allows the transfer of the specificity into T cells isolated from patients that share the TCR MHC-restricting allele. Such T cells could then be expanded and used in adoptive transfer settings to introduce the tumor antigen specificity into patients carrying tumors that express the antigen. T cell receptor alpha and beta chains from a CD8 T cell clone specific for the prostate-specific antigen P501S were isolated and sequenced as follows.

Total mRNA from 2×10^6 cells from CTL clone 4E5 (described above in Example 12) was isolated using Trizol reagent and cDNA was synthesized. To determine Va and Vb sequences in this clone, a panel of Va and Vb subtype-specific primers was synthesized and used in RT-PCR reactions with cDNA generated from each of the clones. The RT-PCR reactions demonstrated that each of the clones expressed a common Vb sequence that corresponded to the Vb7 subfamily. Furthermore, using cDNA generated from the clone, the Va sequence expressed was determined to be Va6. To clone the full TCR alpha and beta chains from clone 4E5, primers were designed that spanned the initiator and terminator-coding TCR nucleotides. The primers were as follows: TCR Valpha-6 5'(sense): GGATCC---GCCGCCACC---ATGTCACITTTCTAGCCTGCT (SEQ ID NO: 899) BamHI site Kozak TCR alpha sequence TCR alpha 3' (antisense): GTCOAC---TCAGCTGGACCACAGCCGCAG (SEQ ID NO: 900) Sall site TCR alpha constant sequence TCR Vbeta-7. 5'(sense): GGATCC---GCCGCCACC---ATGGGCTGCAGGCTGCTCT (SEQ ID NO: 901) BamHI site Kozak TCR alpha sequence TCR beta 3' (antisense): GTCGAC---TCAGAAATCCTTTCTCTTGAC (SEQ ID NO: 902) Sall site TCR beta constant sequence. Standard 35 cycle RT-PCR reactions were established using cDNA synthesized from the CTL clone and the above primers, employing the proofreading thermostable polymerase PWO (Roche, Nutley, NJ).

The resultant specific bands (approx. 850 bp for alpha and approx. 950 for beta) were ligated into the PCR blunt vector (Invitrogen) and transformed into *E. coli*. *E. coli* transformed with plasmids containing full-length alpha and beta chains were identified, and large scale preparations of the corresponding plasmids were generated. Plasmids containing full-length TCR alpha and beta chains were submitted

for sequencing. The sequencing reactions demonstrated the cloning of full-length TCR alpha and beta chains with the determined cDNA sequences for the Vb and Va chains being shown in SEQ ID NO: 903 and 904, respectively. The corresponding amino acid sequences are shown in SEQ ID NO: 905 and 906, respectively. The Va sequence was shown by nucleotide sequence alignment to be 99% identical (347/348) to Va6.2, and the Vb to be 99% identical to Vb7 (336/338).

EXAMPLE 26

CAPTURE OF PROSTATE SPECIFIC CELLS USING

10 THE PROSTATE ANTIGEN P503S

As described above, P503S is found on the surface of prostate cells. Secondary coated microsphere beads specific for mouse IgG were coupled with the purified P503S-specific monoclonal antibody 1D12. The bound P503S antibody was then used to capture HEK cells expressing recombinant P503S. This provides a model system for prostate-specific cell capture which may be usefully employed in the detection of prostate cells in blood, and therefore in the detection of prostate cancer.

P503S-transfected HEK cells were harvested and redissolved in wash buffer (PBS, 0.1% BSA, 0.6% sodium citrate) at an appropriate volume to give at least 5⁴ cells per sample. Round bottom Eppendorf tubes were used for all procedures involving beads. The stock concentrations were as shown below in Table VIII.

Table VIII

Stock concentrations	Sample concentration	Amount needed
Epithelial enrich beads 4 ⁸ beads/ml (Dynal Biotech Inc. Lake Success, NY)	1 ⁷ beads/ml	125 ul stock per 5 ml volume
1D12 ascites antibody 2 mg/ml	0.1 ug/ml (0.1X) to 5 ug/ml (5X) titrations	0.05 ul to 2.5 ul stock per sample
α -Mamma Mu 0.9 mg/ml	1 ug/ml (1X)	1.1 ul stock per sample
Pan-mouse IgG beads 4 ⁸ beads/ml (Dynal Biotech)	1 ⁷ beads/ml	125 ul stock per 5 ml volume

Blocked immunomagnetic beads were pre-washed as follows: all beads needed were pooled and washed once with 1 ml wash buffer. The beads were resuspended in a 3X volume of 1% BSA (v/v) in wash buffer and incubated for 15 min rotating at 4 °C. The beads were then washed three times with 2X volume of wash
5 buffer and resuspended to original volume. Non-blocked beads were pooled, washed three times with 2X volume of wash buffer and resuspended to original volume.

Primary antibody was incubated with secondary beads in a fresh Eppendorf for 30 minutes, rotating at 4 °C. Approximately 200 ul wash buffer was added to increase the total volume for even mixing of the sample. The antibody-bead
10 solution was transferred to a fresh Eppendorf, washed twice with an equal volume of wash buffer and resuspended to original volume. Target cells were added to each sample and incubated for 45 minutes, rotating at 4 °C. The tubes were transferred to a magnet, the supernatant removed, taking care not to agitate the beads, and the samples were washed twice with 1 ml wash buffer. The samples were then ready for RT-PCR
15 using a Dynabeads mRNA direct microkit (Dynal Biotech).

Epithelial cell enrichment was placed in a magnet and supernatant was removed. The epithelial enrichment beads were then resuspended in 100 ul lysis/binding buffer fortified with Rnasin (2 U/ul per sample), and stored at -70 °C until use. Oligo (dT₂₅) Dynabeads were pre-washed as follows: all beads needed were pooled (23
20 ul/sample), washed three times with an excess volume of lysis/binding buffer, and resuspended to original volume. The lysis supernatant was separated with a magnet and transferred to a fresh Eppendorf. 20 ul oligo(dT₂₅) Dynabeads were added per sample and rolled for 5 min at room temperature. Supernatant was separated using a magnet and discarded, leaving the mRNA annealed to the beads. The bead/mRNA
25 complex was washed with buffer and resuspended in cold Tris-HCl.

For RT-PCR, the Tris-HCl supernatant was separated and discarded using MPS. For each sample containing 1⁵ cells or less, the following was added to give a total volume of 30 ul: 14.25 ul H₂O; 1.5 ul BSA; 6 ul first strand buffer; 0.75 mL 10 mM dNTP mix; 3 ul Rnasin; 3 ul 0.1M dTT; and 1.5 ul Superscript II. The resulting
30 solution was incubated for 1 hour at 42 °C, diluted 1:5 in H₂O, heated at 80°C for 2 min

to detach cDNA from the beads, and immediately placed on MPS. The supernatant containing cDNA was transferred to a new tube and stored at -20°C .

Table IX shows the percentage of capture of P503S-transfected HEK cells as determined by RT-PCR.

5

Table IX

	% capture P503S-transfected HEK cells	% capture LnCAP cells
0.1 ug/ml P503S Mab	36.90	0.00
0.5 ug/ml P503S Mab	67.40	2.93
1 ug/ml P503S Mab	40.22	0.00
5 ug/ml P503S Mab	13.11	0.00
Anti-Mu beads only, non-blocked	1.42	0.00
Anti-Mu beads only, blocked	15.65	20.21
Absolute control, non-capture cells	100.00	100.00

10

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

What is Claimed:

1. An isolated polynucleotide comprising a sequence selected from the group consisting of:

(a) sequences provided in SEQ ID NO: 1-111, 115-171, 173-175, 177, 179-305, 307-315, 326, 328, 330, 332-335, 340-375, 381, 382 and 384-476, 524, 526, 530, 531, 533, 535, 536, 552, 569-572, 587, 591, 593-606, 618-705, 709-774, 777, 789, 817, 823, 824, 878, 880-882, 894, 896, 907, 908, 916-919, 929-931, 938, 939 and 942;

(b) complements of the sequences provided in SEQ ID NO: 1-111, 115-171, 173-175, 177, 179-305, 307-315, 326, 328, 330, 332-335, 340-375, 381, 382 and 384-476, 524, 526, 530, 531, 533, 535, 536, 552, 569-572, 587, 591, 593-606, 618-705, 709-774, 777, 789, 817, 823, 824, 878, 880-882, 894, 896, 907, 908, 916-919, 929-931, 938, 939 and 942;

(c) sequences consisting of at least 20 contiguous residues of a sequence provided in SEQ ID NO: 1-111, 115-171, 173-175, 177, 179-305, 307-315, 326, 328, 330, 332-335, 340-375, 381, 382 and 384-476, 524, 526, 530, 531, 533, 535, 536, 552, 569-572, 587, 591, 593-606, 618-705, 709-774, 777, 789, 817, 823, 824, 878, 880-882, 894, 896, 907, 908, 916-919, 929-931, 938, 939 and 942;

(d) sequences that hybridize to a sequence provided in SEQ ID NO: 1-111, 115-171, 173-175, 177, 179-305, 307-315, 326, 328, 330, 332-335, 340-375, 381, 382 and 384-476, 524, 526, 530, 531, 533, 535, 536, 552, 569-572, 587, 591, 593-606, 618-705, 709-774, 777, 789, 817, 823, 824, 878, 880-882, 894, 896, 907, 908, 916-919, 929-931, 938, 939 and 942 under moderately stringent conditions;

(e) sequences having at least 75% identity to a sequence of SEQ ID NO: 1-111, 115-171, 173-175, 177, 179-305, 307-315, 326, 328, 330, 332-335, 340-375, 381, 382 and 384-476, 524, 526, 530, 531, 533, 535, 536, 552, 569-572, 587, 591, 593-606, 618-705, 709-774, 777, 789, 817, 823, 824, 878, 880-882, 894, 896, 907, 908, 916-919, 929-931, 938, 939 and 942;

(f) sequences having at least 90% identity to a sequence of SEQ ID NO: 1-111, 115-171, 173-175, 177, 179-305, 307-315, 326, 328, 330, 332-335, 340-375, 381, 382 and 384-476, 524, 526, 530, 531, 533, 535, 536, 552, 569-572, 587, 591, 593-606, 618-705, 709-774, 777, 789, 817, 823, 824, 878, 880-882, 894, 896, 907, 908, 916-919, 929-931, 938, 939 and 942; and

(g) degenerate variants of a sequence provided in SEQ ID NO: 1-111, 115-171, 173-175, 177, 179-305, 307-315, 326, 328, 330, 332-335, 340-375, 381, 382 and 384-476, 524, 526, 530, 531, 533, 535, 536, 552, 569-572, 587, 591, 593-606, 618-705, 709-774, 777, 789, 817, 823, 824, 878, 880-882, 894, 896, 907, 908, 916-919, 929-931, 938, 939 and 942.

2. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) sequences recited in SEQ ID NO: 112-114, 172, 176, 178, 327, 329, 331, 336, 339, 376-380, 383, 477-483, 496, 504, 505, 519, 520, 522, 525, 527, 532, 534, 537-551, 553-568, 573-586, 588-590, 592, 706-708, 775, 776, 778, 780, 781, 811, 814, 818, 826, 827, 853, 855, 858, 860-862, 866-877, 879, 883-893, 895, 897, 898, 909-915, 920-928, 932-934, 940, 941 and 943;

(b) sequences having at least 70% identity to a sequence of SEQ ID NO: 112-114, 172, 176, 178, 327, 329, 331, 336, 339, 376-380, 383, 477-483, 496, 504, 505, 519, 520, 522, 525, 527, 532, 534, 537-551, 553-568, 573-586, 588-590, 592, 706-708, 775, 776, 778, 780, 781, 811, 814, 818, 826, 827, 853, 855, 858, 860-862, 866-877, 879, 883-893, 895, 897, 898, 909-915, 920-928, 932-934, 940, 941 and 943;

(c) sequences having at least 90% identity to a sequence of SEQ ID NO: 112-114, 172, 176, 178, 327, 329, 331, 336, 339, 376-380, 383, 477-483, 496, 504, 505, 519, 520, 522, 525, 527, 532, 534, 537-551, 553-568, 573-586, 588-590, 592, 706-708, 775, 776, 778, 780, 781, 811, 814, 818, 826, 827, 853, 855, 858, 860-862, 866-877, 879, 883-893, 895, 897, 898, 909-915, 920-928, 932-934, 940, 941 and 943;

(d) sequences encoded by a polynucleotide of claim 1;

(e) sequences having at least 70% identity to a sequence encoded by a polynucleotide of claim 1; and

(f) sequences having at least 90% identity to a sequence encoded by a polynucleotide of claim 1.

3. An expression vector comprising a polynucleotide of claim 1 operably linked to an expression control sequence.

4. A host cell transformed or transfected with an expression vector according to claim 3.

5. An isolated antibody, or antigen-binding fragment thereof, that specifically binds to a polypeptide of claim 2.

6. A method for detecting the presence of a cancer in a patient, comprising the steps of:

(a) obtaining a biological sample from the patient;

(b) contacting the biological sample with a binding agent that binds to a polypeptide of claim 2;

(c) detecting in the sample an amount of polypeptide that binds to the binding agent; and

(d) comparing the amount of polypeptide to a predetermined cut-off value and therefrom determining the presence of a cancer in the patient.

7. A fusion protein comprising at least one polypeptide according to claim 2.

8. An oligonucleotide that hybridizes to a sequence recited in SEQ ID NO: 1-111, 115-171, 173-175, 177, 179-305, 307-315, 326, 328, 330, 332-335, 340-375, 381, 382 and 384-476, 524, 526, 530, 531, 533, 535, 536, 552, 569-572, 587, 591,

593-606, 618-705, 709-774, 777, 789, 817, 823, 824, 878, 880-882, 894, 896, 907, 908, 916-919, 929-931, 938, 939 and 942 under moderately stringent conditions.

9. A method for stimulating and/or expanding T cells specific for a tumor protein, comprising contacting T cells with at least one component selected from the group consisting of:

- (a) polypeptides according to claim 2;
- (b) polynucleotides according to claim 1; and
- (c) antigen-presenting cells that express a polypeptide according to claim 2,

under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells.

10. An isolated T cell population, comprising T cells prepared according to the method of claim 9.

11. A composition comprising a first component selected from the group consisting of physiologically acceptable carriers and immunostimulants, and a second component selected from the group consisting of:

- (a) polypeptides according to claim 2;
- (b) polynucleotides according to claim 1;
- (c) antibodies according to claim 5;
- (d) fusion proteins according to claim 7;
- (e) T cell populations according to claim 10; and
- (f) antigen presenting cells that express a polypeptide according to claim 2.

12. A method for stimulating an immune response in a patient, comprising administering to the patient a composition of claim 11.

13. A method for the treatment of a cancer in a patient, comprising administering to the patient a composition of claim 11.

14. A method for determining the presence of a cancer in a patient, comprising the steps of:

- (a) obtaining a biological sample from the patient;
- (b) contacting the biological sample with an oligonucleotide according to claim 8;
- (c) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; and
- (d) compare the amount of polynucleotide that hybridizes to the oligonucleotide to a predetermined cut-off value, and therefrom determining the presence of the cancer in the patient.

15. A diagnostic kit comprising at least one oligonucleotide according to claim 8.

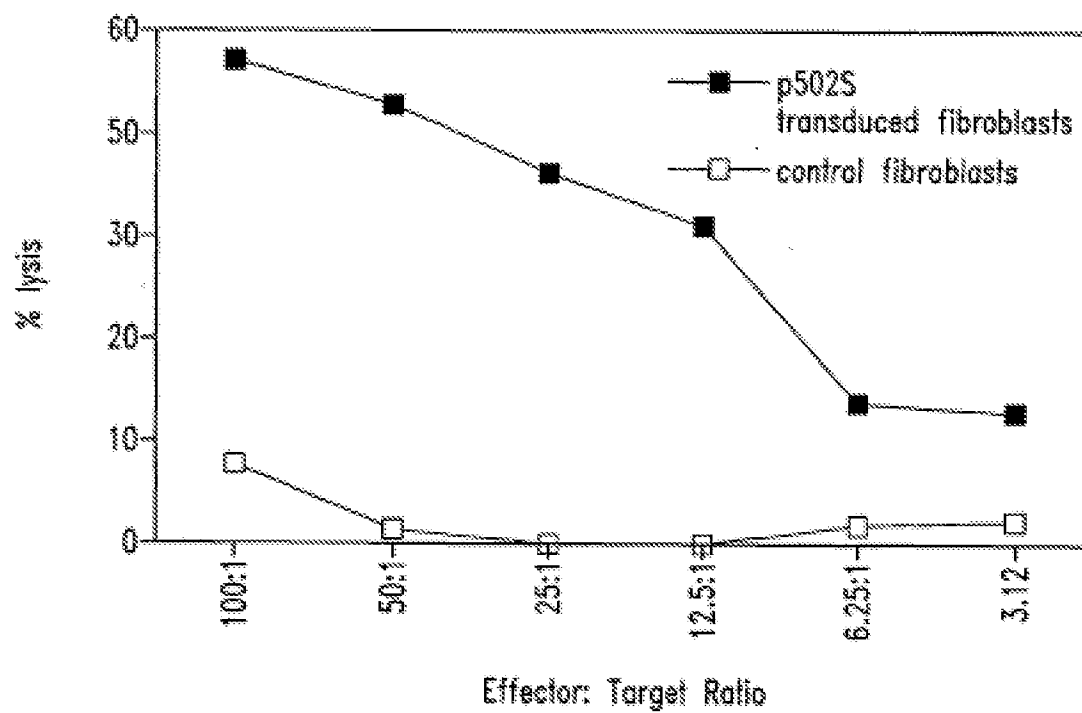
16. A diagnostic kit comprising at least one antibody according to claim 5 and a detection reagent, wherein the detection reagent comprises a reporter group.

17. A method for inhibiting the development of a cancer in a patient, comprising the steps of:

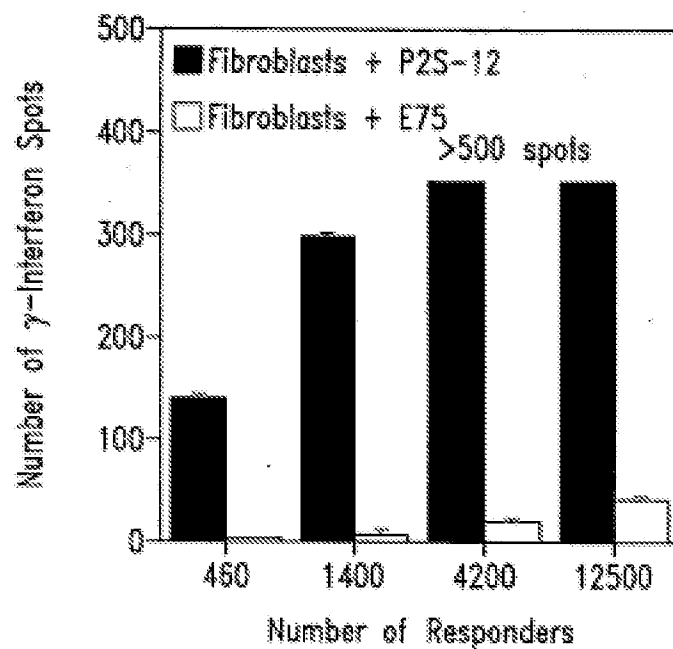
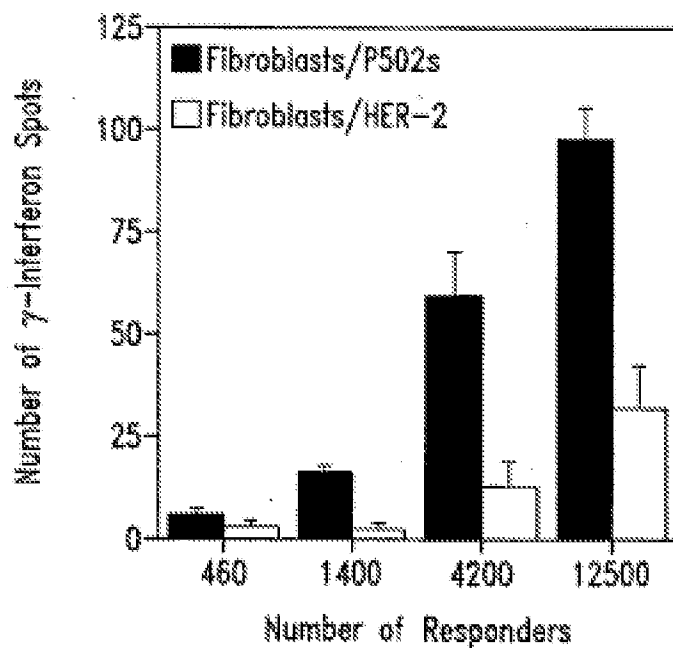
- (a) incubating CD4+ and/or CD8+ T cells isolated from a patient with at least one component selected from the group consisting of: (i) polypeptides according to claim 2; (ii) polynucleotides according to claim 1; and (iii) antigen presenting cells that express a polypeptide of claim 2, such that T cell proliferate;
- (b) administering to the patient an effective amount of the proliferated T cells,

and thereby inhibiting the development of a cancer in the patient.

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*Fig. 1*

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*Fig. 2A**Fig. 2B*

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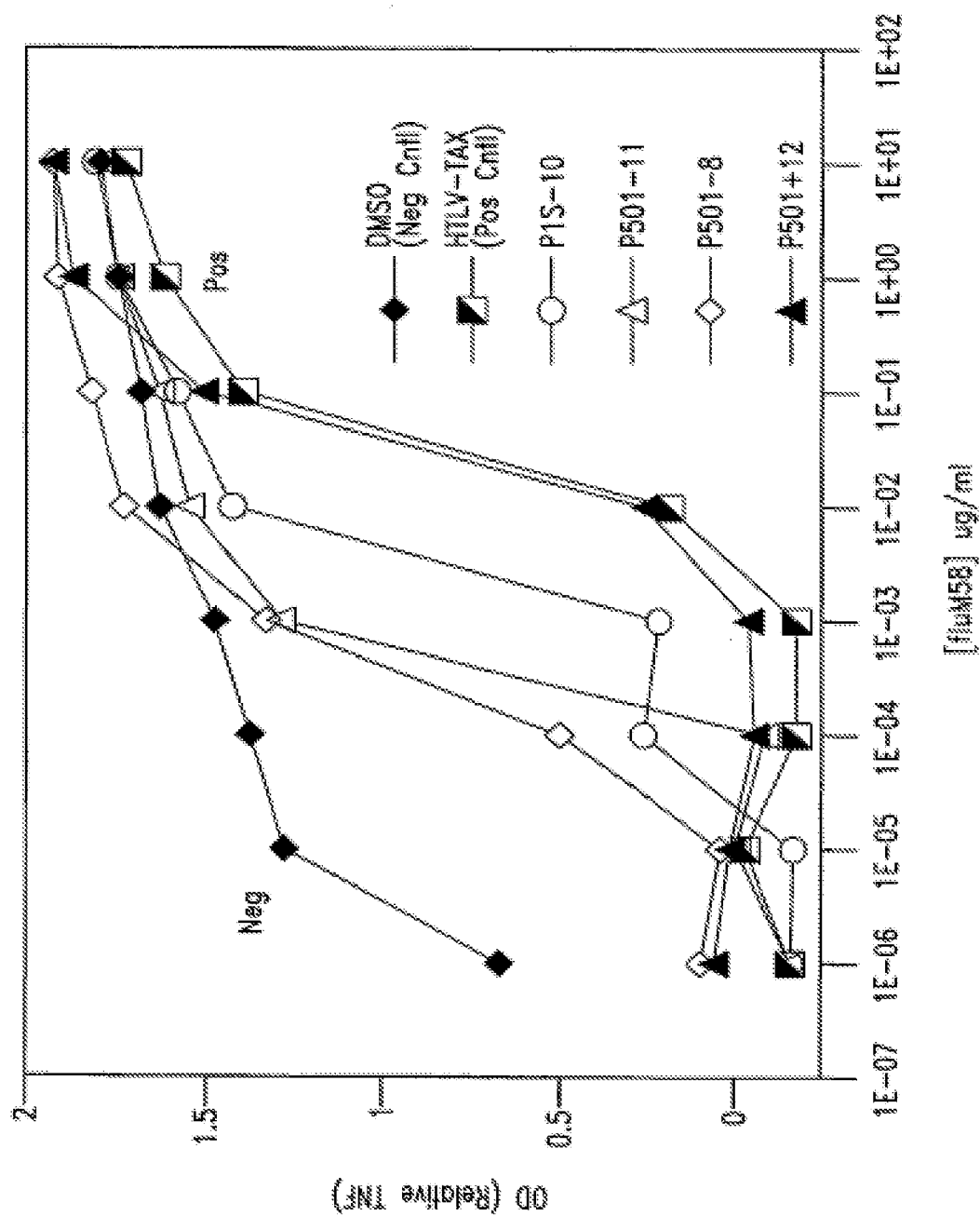
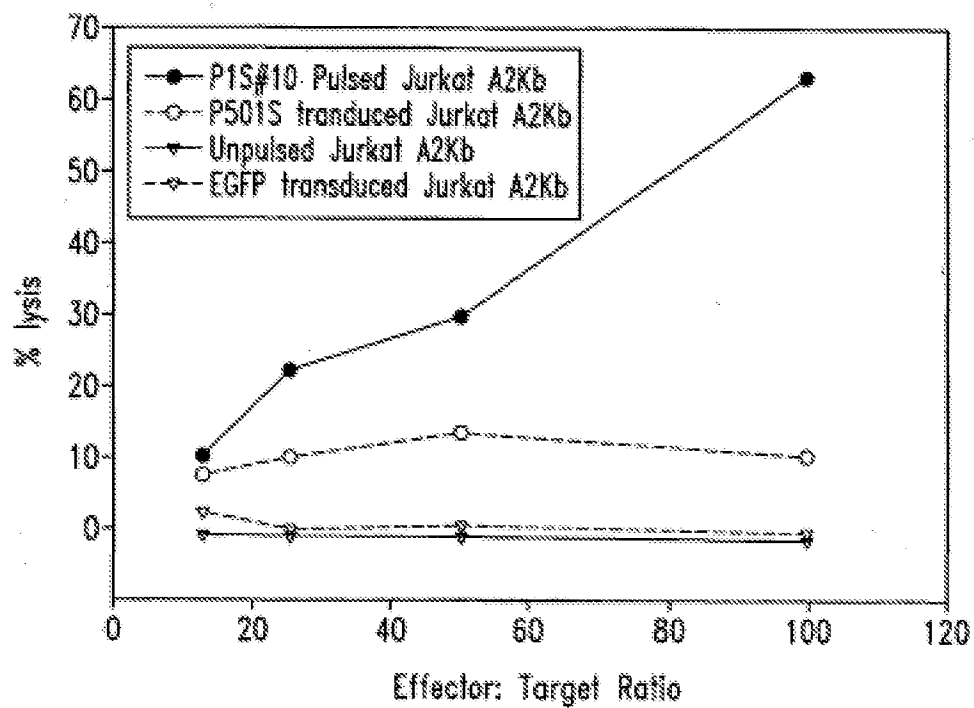
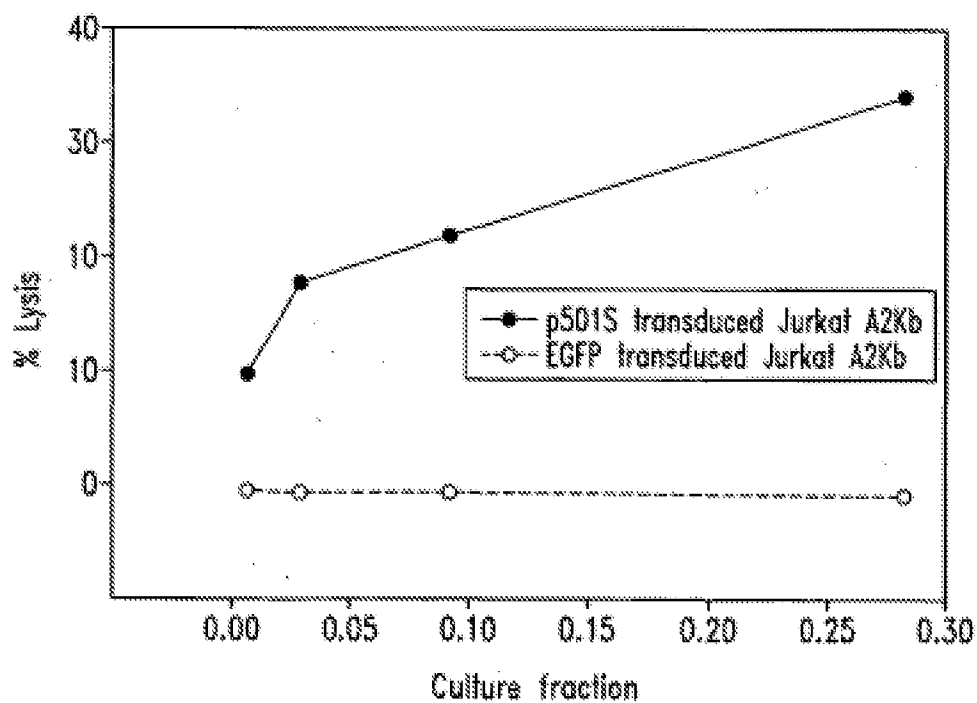
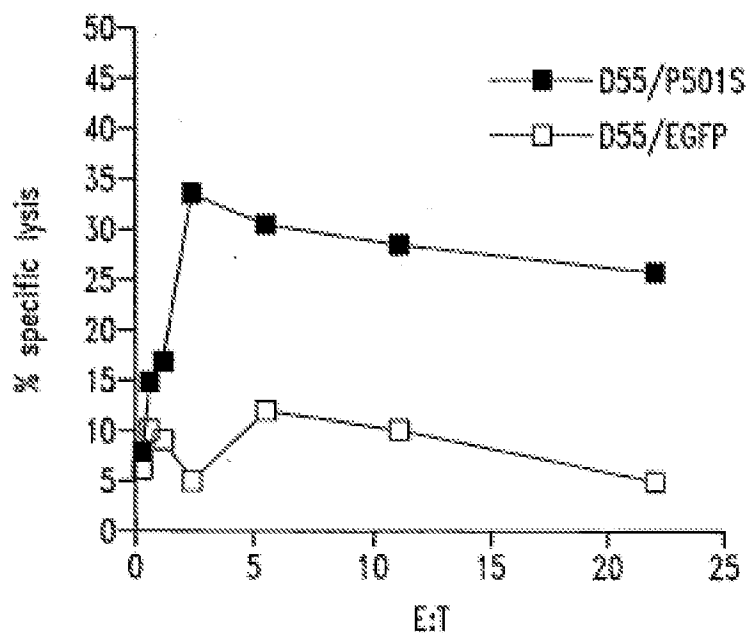
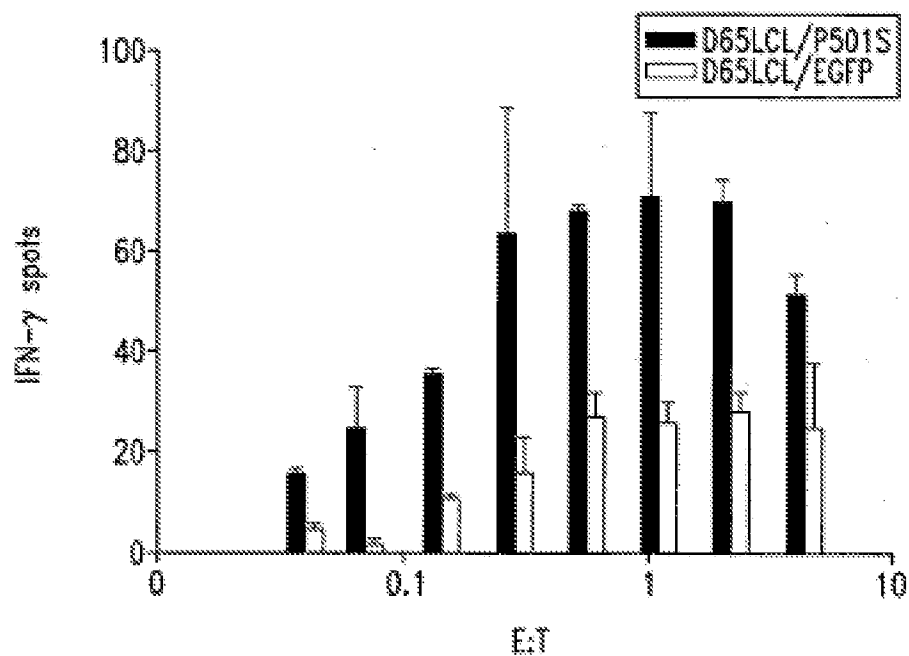


Fig. 3

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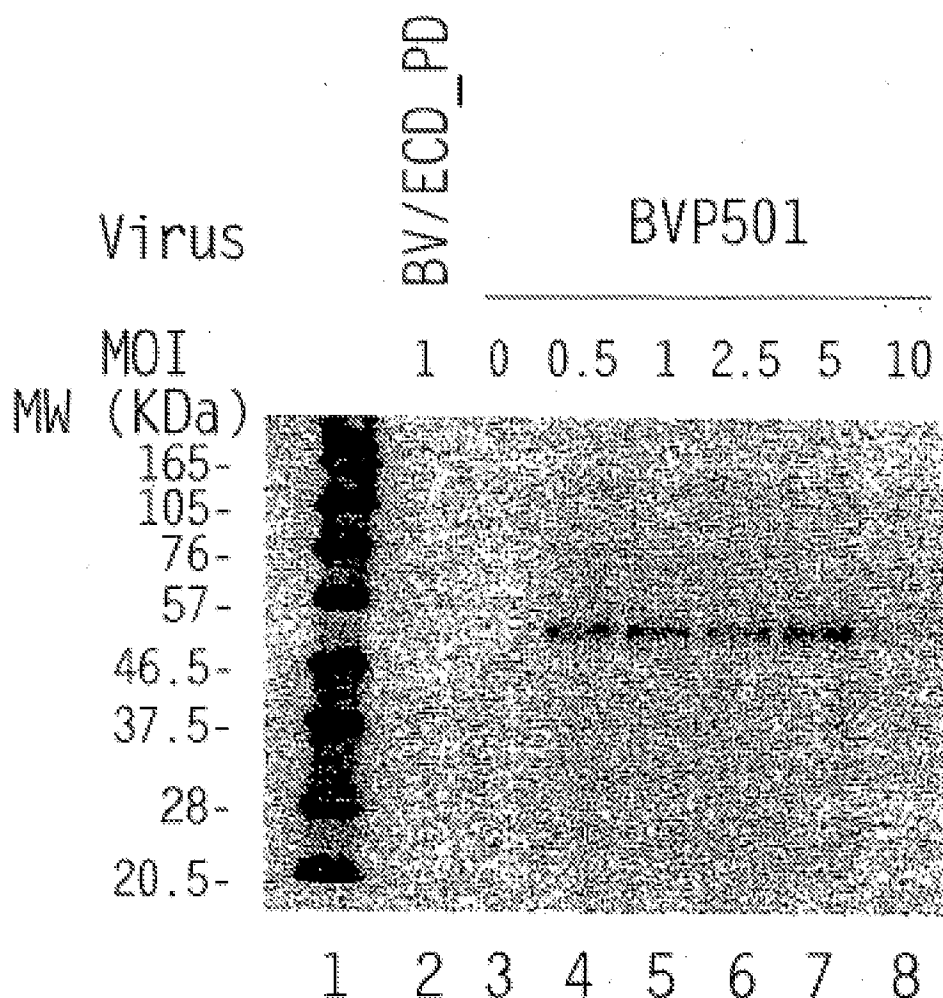
*Fig. 4**Fig. 5*

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*Fig. 6A**Fig. 6B*

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Expression of P501S
by the Baculovirus Expression System



0.6 million high 5 cells in 6-well plate were infected with an unrelated control virus BV/ECD_PD (lane2), without virus (lane3), or with recombinant baculovirus for P501 at different MOIs (lane 4-8). Cell lysates were run on SDS-PAGE under the reducing conditions and analyzed by Western blot with a monoclonal antibody against P501S (P501S-10E3-G4D3). Lane 1 is the biotinylated protein molecular weight marker (BioLabs).

Fig. 7.

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FIGURE 8. Mapping of the epitope recognized by 10E3-G4-D3

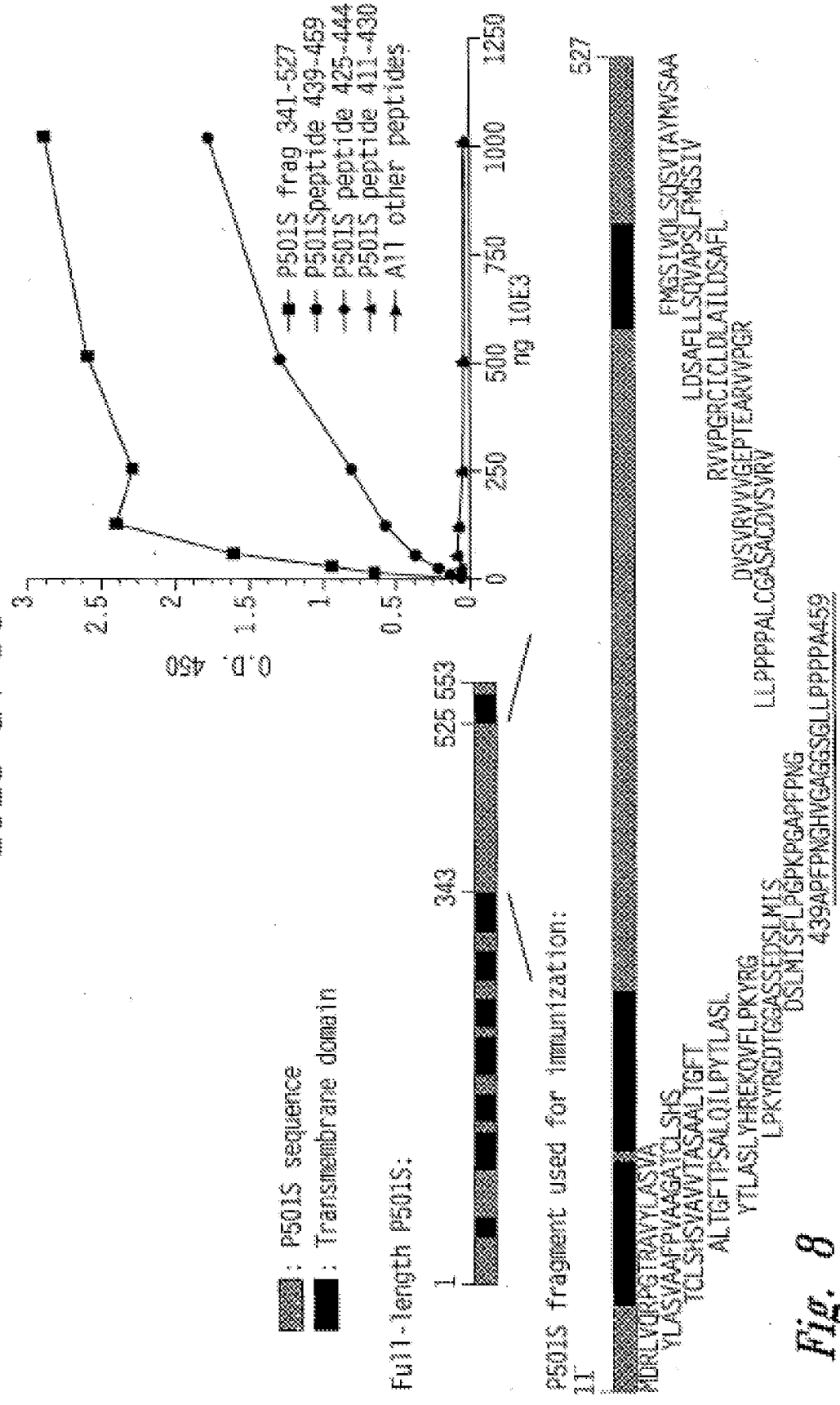


Fig. 8

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Schematic of P501S with predicted
transmembrane, cytoplasmic, and extracellular regions

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HQLCCRMPTLR LFVAELCSWALMTFTLFYTD VGEGLYQGVPRAEPTARRHYDEGVR

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LPPPPALCGASACDVSVRVVVGEPTEARVVPGRG ICLDLAILDSAFLLSQVAPSLF MGSIVQLSQS

VTAYMVSAAGLGLVATYFAT QVVFDKSDLAKYSA

Underlined sequence: Predicted transmembrane domain; **Bold sequence**:
Predicted extracellular domain; *Italic sequence*: Predicted intracellular
domain. Sequence in bold/underlined: used generate polyclonal rabbit
serum

Localization of domains predicted using HMMTOP (G.E. Tusnady and I. Simon
(1998) Principles Governing Amino Acid Composition of Integral Membrane
Proteins: Applications to topology Prediction. J. Mol Biol. 283, 489-506.

Fig. 9

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Genomic Map of (5) Corixa Candidate Genes

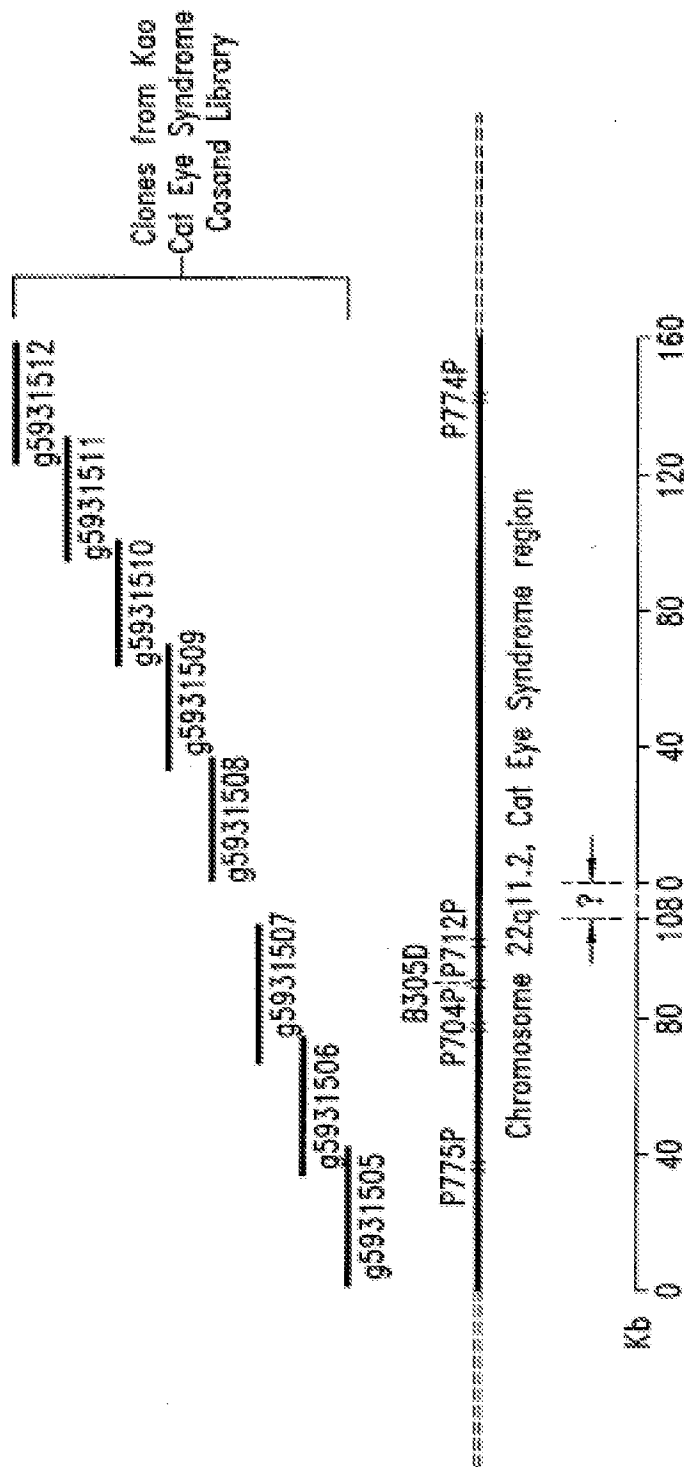


Fig. 10

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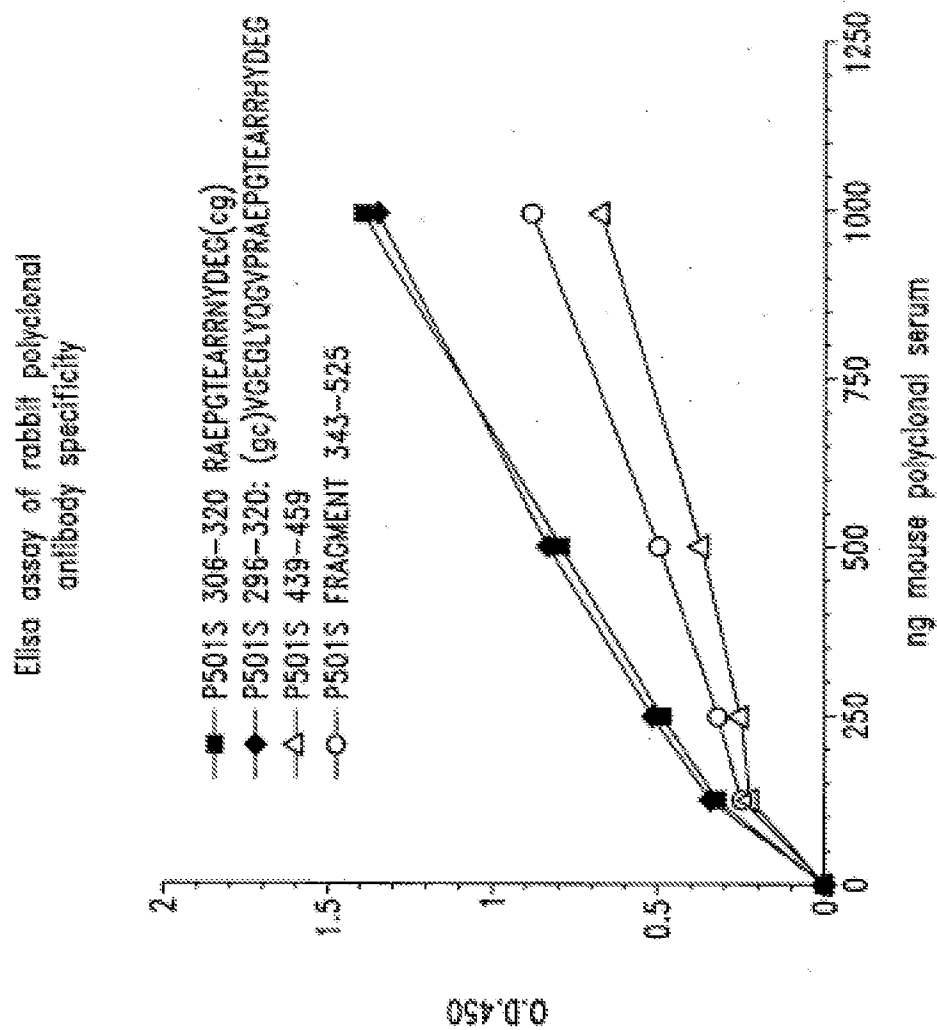


Fig. 11

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Fig. 12A (1)

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Fig. 12A (2)

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Fig. 12A (3)

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Fig. 12B

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 Harlocker, Susan L.
 Yuqui, Jiang
 Kalos, Michael D.
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 Stolk, John A.
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 tctgccttcg tcttctttgc aaatacatct gcaaacctct tcttcatttc tggccaatca 240
 tccatgctca tctgattggg aagttcatca gactttagtc canntccttt gatcagcagc 300
 tegtagaact ggggttctat tgcctcaaca gccatgaatt ccccatctgc tgtcctgtaa 360
 gtcttataga aaggtgctcc accatccaac atgttctgtc ctgcaggggg ggcgcgtac 420
 ccaattcgcc ctatantgag tegtattacg cgcgctcaat ggcgctcgtt ttacaacgtc 480
 gtgactggga aaacccctgg cgttaaccaac ttaactgcct tgcagacat ccccttttcg 540
 ccagctggga gtaatanaga aaagggccgc accgatcgcc attccaaacag ttgcgcacct 600
 gaattgggaa atgggacccc cctgttacgc cgcattnaac ccccgcnagg ttngttgtt 660
 accccacnt nnaccgetta caatttgcac gcgccttanc gccgcctccc tttncccttt 720
 ctcccttcc tttnccnccn ctttccccc ggggttcccc cttcaaaccc cna 773

<210> 4
 <211> 828
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(828)
 <223> n = A,T,C or G

<400> 4
 cctcctgagt cctactgacc tgtgctttct ggtgtggagt ccagggctgc taggaaaagg 60
 aatgggcaga cacaggtgta tgccaatgtt tctgaatgg gtataatttc gtctctctct 120
 tcggaacact ggcctgtctct gaagacttct cgcctcagtt cagtgaggac acacacaaag 180
 acgtgggtga ccatgttctt tgtgggtgc agagatggga ggggtggggc ccaccctgga 240
 agagtggaca gtgacacaag gtggacactc tctacagatc actgaggata agctggagcc 300
 acaatgcctg aggcacacac acagcaagga tgacnctgta aacatagccc acgtgtctct 360
 gngggcactg ggaagcctan atraggccgt gagcanasag aaggggagga tccactagtt 420
 ctanaggggc cgcacacggg gtgganctcc ancttttgtt ccttttagtg agggttaat 480
 gogogcttgg cmtaatctg gtctatnctn tttcctgtgt gaaattgta tccgtcaca 540
 attccacaca acatacganc cggaaacata aantgtaaac ctgggggtgc taatgantga 600
 cttaactaca ttaattgctt tgcgtcact gcccgcttcc caatcnggaa acctgtcttg 660
 ccncttgcat tnatgaatcn gccaaccccc ggggaaaaagc gtttgctttt tgggcgtctt 720
 tccgcttctt cnetcantta ntccctnccn tcgggtcatt ccggtgcnge aaaccggttc 780
 acnctctca aagggggtat tccggttcc ccaatccgg gganance 828

<210> 5
 <211> 834
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(834)
 <223> n = A,T,C or G

<400> 5
 tttttttttt tttttactga tagatggaat ttattaagct ttccacatgt gatagcacat 60
 agtttttaatt gcatccaaag taactaacaa aactctagca atcaagaatg gcagcatgtt 120
 atttttataac aatcaacacc tgtggctttt aaaatttggt ttctataaga taatttatcc 180
 tgaagttaat cttagcatgc ttttaaaaaa tgccttaggt cactccaage ttggcagtta 240
 acattttgga taacaataa taacaacatc acaatttaat aaataacaaa tacaacattg 300
 taggccataa tcatatcacg tataaggaaa aggtggtagt gttgagtaag cagttatttag 360
 aatagaatac cttggcctct atgcaaatat gtctagacac tttgattcac tcagccctga 420
 cattcagttt tcaaaqtagy agacaggttc tccagtatca ttttacagtt tccaacacat 480
 tgaaaacsag tagaaaatga tgagttgatt tttattaatg cattacatcc tcagagttta 540
 tcaccaaccc ctcagttata aaaaatttcc aagtttatatt agtcatatca cttgggtgtc 600
 ttatttttaa ttagtgctaa atggattaa tgaagacaa aatgggtccc taatgtgatt 660
 gatatttggtc atttttacca gottctaaat ctnaacttcc aggtttttga actggaacat 720
 tgnatnacag tgttccanag ttncaacctc ctggascatt acagtgctgt tgattcaaaa 780
 tgttattttg ttaaaaatta aattttaacc tgggtgaaaa ataatttga atna 834

<210> 6
 <211> 810
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(810)
 <223> n = A,T,C or G

<400> 6

```

tttttttttt tttttttttt aagacccctc tcaatagatg gagacataca gaaatagtca      60
aaccacatct acaaaatgcc agtatcaggg ggcggcttcg aagccaaagt gatgttttga      120
tgtaaaagtga aatattagtt ggcggatgaa gcagatagtg aggaaggttg agccaataat      180
gacgtgaagt cegtggagac ctgtggctac aaaaaatgtt gagccgtaga tgccctcgga      240
aatggtagag ggagactcga agtactctga ggcttgtagg agggtaaaat agagacccag      300
taaaattgta ataagcagtg cttgaattat ttggtttcgg ttgttttcta tttagactatg      360
gtgagctcag gtgattgata ctccctgatg gagttaatac gatgtgttta ggaagtgggc      420
ttctagggga tttagcgggg tgatgacctg tggggggccag tgccctccta gttggggggt      480
aggggctagg ctggagtggt aaaaggctca gaaaaatcct gcgaagaaaa aaacttctga      540
ggtaataaat aggattatcc cgtatcgagc gccttttttg acaggttggtg tgtggtggcc      600
ttggtatgtg ctttctcgtg ttacatcgcg ccatcattcg tatatggtta gtgtgttggy      660
ttantanggc ctantatgaa gaaetttttg antggaatta aatcaatngc ttggccggaa      720
gtcattanga nngctnaaaa ggcctgttta agggctctgg ctnggtttta cccnaccat      780
ggaataacac ccccggaana ntgnatccct attcttaa      816

```

<210> 7
 <211> 817
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(817)
 <223> n = A,T,C or G

```

<400> 7
tttttttttt tttttttttt tggctctaga gggggtagag ggggtgctat agggtaata      60
cgggccctat ttcaaaagatt tttaggggaa ttaattctag gcgatgggt atgaacctgt      120
ggtttgctcc acagatttca gagcattgac cgtagtatac ccccggtcgt gttagcgtga      180
aagtggtttg gtttagaagt cggggaattg catctgtttt taagccataat gtggggacag      240
ctcatgagtg caagacgtct tgtgatgtaa ttattatacn aatgggggct tcaatcggga      300
gtactactcg attgtcaacg tcaaggagtc gcaggtcgcc tggttctagg aataatgggy      360
gaagtatgta ggaattgaag attaatccgc cgtagtccgt gttctcctag gttcaatacc      420
attggtggcc aattgatttg atggtaaggg gagggatcgt tgaactcgtc tgttatgtaa      480
aggatnccct nggatggga agcnaatnaa ggactangga tnaatggcgg gcangatat      540
tcaaacngtc tctanttcct gaaacgtctg aaatgttaat aanaattaan tttngttatt      600
gaatnttng gaaaagggct tacaggacta gaaaccaaat angaaaanta atntaanggy      660
cattatenta aaagginata accnctccta tnatccacc ccatngnatl cccacnccn      720
acnattgat nccccattc canaaanggc cccccccgg tgnannccnc cttttgttcc      780
cttnaatgan ggttattenc cctngcatt atcanc      817

```

<210> 8
 <211> 799
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(799)
 <223> n = A,T,C or G

```

<400> 8
catttcggg tttactttct aaggaaagcc gagcggagc tgcataacgt ggaatcggtg      60
cataaggaga attttctgct ggcacggcct agggacaagc gggagagcga ctccgagcgt      120
ctgaagcgca cgtcccagaa ggtggacttg gcactgaaac agctgggaca catcccgag      180
taogaacaga goctgaaggt gctggagcgg gaggtccagc agtgtagccg cgtcctgggg      240
tgggtggcgg angcctganc cgtctctgct tgetgcccc angtgggccg cccccccctg      300
acctgctgg gtccaaacac tgagccctgc tggcggactt caagganaac cccccangg      360

```

```

ggatttttgcct cctanantaa ggtctatctg ggctctggcc cccccacctg gttggacctg 420
tcttttgangt gagccccatg tccatctggg ccaetgtcng gaccaccttt ngggagtgtt 480
ctcettacaa ccacannatg cccggtctct cccggaaacc antccancc tnggaaggat 540
caagnctgn atccactnnt nctanaacog gcncncncog cngtggaaac cnccttntgt 600
tcttttctnt tnagggttaa tnnogccttg gocttnccan ngctctnnc nttttccnt 660
gttnaaattg ttangcncc nccntcccn cncncncan cccgaccan annttannan 720
nctgggggt ncnncngat tgaccnncc nccctntat tgcnttggg ncnntgccc 780
cttccctct nggganncc

```

<210> 9

<211> 801

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(801)

<223> n = A,T,C or G

<400> 9

```

acgccttgat cctccacaggc tgggaactggt tctgggagga gcggggcatg ctgtggtttg 60
taangatgac actcccaag gtggtctga cagtggccca gatggacatg gggtccact 120
caaggacaaag gccaccaggt gcggggggccg aagccacat gatccttact ctatgagcaa 180
aatccctgt gggggcttct ccttgaagtc cggcancagg gctcagttt tggaccang 240
caggtcatgg ggttctngnc caactgggg cncacacga aaanggcna gggcctcngn 300
caccatccc angaogggc tacactnctg gacctccnc tccaccactt tcatgogctg 360
ttentaccc cgnatnctc ccanctgtt cngtgcncac tccancttct nggaogtgg 420
ctacatacgc cggantcnc nctcccgctt tgtccctatc caogtnccan caacaaattt 480
cncctantg caccnattcc caontttnc agntttcnc nncngcttc ctntaaaaag 540
ggttganccc cggaaaatnc cccaaagggg gggggccng taccacactn cccctnata 600
gctgaantcc ccatnccnn gactcnatgg anccntcct ttaannacc ttctnaacti 660
gggaananc ctcgnccntn ccccnctta tccnccctg cnangmnt ccccnctcc 720
ncccnatng gcntatnann cnaaaaaggg cennanaca tctctnncn cctcanttgg 780
ccnccctcg aatcggcnc c

```

<210> 10

<211> 789

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(789)

<223> n = A,T,C or G

<400> 10

```

cagttatnt ggccagtgtg gcagctttcc ctgtggctgc cgtgcccaca tgcctgtccc 60
acagtgtggc cgtggtgaca gtttcagccg cctccaccgg gtccaccttc tcagccctgc 120
agatcctgac ctacacactg gctccctct accaccggga gaagcaggtg tctctgccc 180
aataccgaag ggaactgga ggtgctagca gtgaggacag cctgatgacc agcttctgc 240
caggecttaa gctggagct ccttcccta atggacact ggtgtgtgga ggcagtggcc 300
tgetccccc tccaccogcg ctctggggg cctctgctg tgatgtctcc gtacgtgtgg 360
tggtgggtga gccaccgan gccagggtag tccggggccg gggcatctgc ctgacctcg 420
ccatcctgga tagtgettec tgetgtccc ngtggeccca tccctgttta tgggctccat 480
tgtccagctc agccagtctg tcactgcta tatggtgtct gccgcaggcc tgggtctggt 540
ccatttact ttgtacaca ggtantatct gacagaagc anttggccaa atactcagcg 600
ttaaaaatt ccagcaacat tgggggtgga aggcctgct cactgggtcc aactccccc 660
tctgttaac ccatggggc tgcggcttg gccgccatt tctgtgtgt ccaantnat 720

```

gtggetctct getgecaact gttgetggct gaagtgcata cngcncanct nggggggtnq 788
ggagttccc 789

<210> 11
<211> 772
<212> DNA
<213> Homo sapien

<220>
<221> misc feature
<222> (1)...(772)
<223> n = A,T,C or G

<400> 11
ccacccctac ccaaatatta gacaccaaca cagaaaagct agcaatggat tcccttctec 60
ttgtttaast aaataagtta aatatttaaa tgcctgtgtc tctgtgatgg caacagaagg 120
accaacagge cacatcctga taaaaggtaa ggggggggtg gatcagcaaa aagacagtgc 180
tgtgggtgta ggggacctgg ttcttgtgtg ttgccctca ggaactcttc cctacaaata 240
acttccatgt gttcaaatcc catggaggag tgtttcatcc tagaaactcc catgcaagag 300
ctacattaaa cgaagctgca ggtlaagggg ctlanagatg ggaacecagg tgactgagtt 360
tattcagctc ccaaaaaccc ttctctaggt gtgtctcaac taggaggcta gctgttaacc 420
ctgagcctgg gtaateccac tgcagagtc cgcattcca gtgcattgaa cctttctggc 480
ctccctgtat aagtccagac tgaacccccc ttggagggnc tccagtcagg cagccctana 540
aactggggaa aaaaagaaaag gacgcccacn cccccagctg tgcactacg cactccaca 600
gcacagggtg gacgcaaaaa aaccacttta ctttggcaca aacaaaaact ngggggggca 660
acccgggac cccnagggg gtaaacagga ancggggnaa cntggaaccc aattnaggea 720
ggcccnccac ccnaatnnt gctgggaaat ttttccccc cttaattntt tc 772

<210> 12
<211> 751
<212> DNA
<213> Homo sapien

<220>
<221> misc feature
<222> (1)...(751)
<223> n = A,T,C or G

<400> 12
gccccaatc cagctgccac accacccacg gtgactgcat tagttcggat gtcatacaaa 60
agctgattga agcaaccctc tactttttgg tctgtagcct ttgtcttggg gcaggtttca 120
ttggctgtgt tgggtacgtt gtcattgcaa cagaatgggg gaaaggcact gttctctttg 180
aagtanggtg agtccctcaa atccgtatag ttggtgaagc cacagcactt gagcccttcc 240
atggtggtgt tccacacttg agtgaagtct tccctgggac cataactctt cttgatggca 300
ggcactacca gcaacgtcag ggaagtgtc agccattgtg gtgtacacca aggegaccac 360
agcagctgcn aactcagcaa tgaagatgan gaggangatg aagaagaacg tencgagggc 420
acacttgctc ttagtcttan caaccatana gcccttysaa accaanana aagaccacna 480
cncggctgc gatgaagaaa tnaaccnccg ttgacaaact tgcattggcc tgggancacc 540
agtggccna aaatcttca aaaggatgc cccatcnatt gaccccccna atgcccactg 600
ccaacagggg ctgcccacn cncnascga tgaacnatt gnacaagatc tncntggtct 660
tnatnaact gaacctgcn tngtggctcc tgttcaggnc cngggcctga cttctnaann 720
aangaactcn gaagncccc cngganann g 751

<210> 13
<211> 729
<212> DNA
<213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(729)
 <223> n = A,T,C or G

<400> 13
 gagccaggcg tccctctgac tgeccactca gtggcaaacac ccggggagctg ttttgtccctt 60
 tgtgganccct cagcagtncc ctctttcaga actcantgoc aaganccttg aacaggagcc 120
 acctgacagt gcttcagctt catlaagacc atgatgatcc tcttcaattt gctcatcttt 180
 ctgtgtggtg cagccctggt ggcagtgggc atctgggtgt caetogatgg ggcctccctt 240
 ctgaagatct tggggccact gtctccagt gccatgcagt ttgtcaacgt gggctacttc 300
 ctcatgcag ccggcggtgt ggtcttagct ctaggtttc tgggtgcta tgggtctaag 360
 actgagagca agtgtgccc cytgacgttc ttcttcaccc tccctccctt ctccattgct 420
 gaggttgcaa tgcgtgtgtt gccttggtgt acaccacaa ggttgagcac ttctgacgt 480
 tgcgtgtaat gcttgccatc aaaaaaagat tatgggttcc cagggaact tcaactcaag 540
 gttggaacac caccatgaaa gggctcaagt gctgtggctt cnnccaacta taaggatttt 600
 gaagantcac ctacttcaaa gaaaanagt cctttccccc atttctgttg caattgacaa 660
 aogtccccc caccagccat tgaanaactg caccacaacc aaangggctc ccaaccanaa 720
 attsaaggg 729

<210> 14
 <211> 816
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(816)
 <223> n = A,T,C or G

<400> 14
 tgetcttctc caaagttgtt ctgtttgcca taacaaccac cataggtaaa ggggggcag 60
 tgttgcctga aggggttcta gtaccagcgc gggatgctct ccttgacagc tctgtgtct 120
 ggcaggtcca ccagtgccc tttgtcactg gggaaatgga tgcgttgag ctctcctcag 180
 ccactgctgt atttttcaca ggcagcctcg tccgacgcgt cggggcaggt ggggggtgt 240
 tcacactcca ggaactgtc natgcagcag ccattgctgc agcggaactg ggtgggctga 300
 cangtgcag agcacactgg atggcgctt tccatgnan gggccctgng ggaagtccc 360
 tgancccca anctgcctct caaangcccc acctgcaca ccccgacag ctagaatgga 420
 atcttcttcc cgaaggtag ttnttcttgt tgeccancc anccccntaa acaactctt 480
 gcanatctgc tccngggggg tentantacc anctgtggaa agaaaccca ggengcagc 540
 caancitgtt tggatncgaa gcnataatct nctattctgc ttggtggaca gcaccantaa 600
 ctgtnnanct ttagnccctg gtctctctgg gttgmettg aacctactn cennatcaat 660
 gggacaaggt aantngcct ccttttaatt cccnancntn cccctggtt tggggtttt 720
 cncctccta cccagaaan nccgtgttcc cccccaacta ggggcnaaa cennatnttc 780
 cacaacctn cccacccac gggttcngnt ggttng 816

<210> 15
 <211> 783
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(783)
 <223> n = A,T,C or G

<400> 15
 ccaaggcctg ggcaggcata naettgaag tacaaccccc ggaacccctg gtgctgaagg 60

```

atgtggasaa cacagattgg cgcctactgc ggggtgacac ggatgtcagg gtagagagga 120
aagaccccaa ccaggtggaa ctgtggggac tcaaggaang cacttacctg ttccagctga 180
cagtgaactag ctcagaccac ccagaggaca cggccaacgt cacagtcact gtgctgtcca 240
ccaagcagac agaagactac tgcctcgcat ccaacaangt gggtcgctgc cggggctctt 300
tcccacgctg gtactatgac cccacggagc agatctgcaa gaggttcgtt tatggaggct 360
gcttgggcaa caagaacacac taccttcggg aagaagagtg cattctance tgtcnggggtg 420
tgaagggtgg gcctttgana nccanctctg gggtcange gactttccc cagggccctt 480
ccatggasag gcgcctatca ntgttctctg gcacctgtca gccacccag ttccgctgca 540
ncaatggctg ctgcctcnac antttctctg aattgtgaca acacccccc ntgtccccc 600
ccctcccaac aaagcttccc tgttcaaaaa tacnccantt ggctttttac aaacnccgg 660
cncctccatt ttcccccatt aacaaagggc nctngccttt gaactgccc aaacnngaa 720
tcnccnngg aaaaantccc cccctgggtt cctnnaance cctccncaa anctncccc 780
ccc 783

```

<210> 16
 <211> 801
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)... (801)
 <223> n = A,T,C or G

```

<400> 16
gcccaaatc cagctgccac accacccacg gtgactgcct tagttcggat gtcatacaaa 60
agctgattga agcaacccct tacttttttg tctgagcct tttgcttggg gcaggtttca 120
ttggctgtgt tgggtgaggt gtcattgcaa cagaatgggg gaaaggcact gttctctttg 180
aagttaggtg agtccctaaa atccgtatag ttggtgaagc cacagcactt gagccctttc 240
atggtgtgtg tccacacttg agtgaagctc tccctgggac cataatcttt ctgtatggca 300
ggcactacca gcaacgtcag gaagtgcct gccattgttg tgtacacca ggcgaccaca 360
gcagctgcaa cctcagcaat gaagatgagg aggaggatga agaagaacgt cncgagggca 420
cacttgcctc cgtctttagc accatagcag cccangaaac caagagcaaa gaccacaacg 480
cncgtgcga atgaagaaaa ntacccacgt tgcaaaactg catggccact ggaagacagt 540
tggcccgaaa atcttcagaa aagggatgac ccatcgattg aacacnana tgcccactgc 600
cncagggctt gncnccnccn gaagaatga gccattgaag aaggatcctc atggtcttaa 660
tgaactgaaa centgcatgg tggccctgt tcaaggctct tggcagtgaa ttctganaaa 720
aaggaaacgc ntnagcccc cccangana aaacaccccc ggggtgttgc ctgaattggc 780
ggccaaggan cctgcccncn g 801

```

<210> 17
 <211> 740
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)... (740)
 <223> n = A,T,C or G

```

<400> 17
gtgagagcca ggcgtccctc tgcctgccea ctcagtggca acacccggga gctgttttgt 60
cctttgtgga gectcagcag ttccctcttt cagaactcac tgccaagagc cctgaacagg 120
agccacccatg cagtgccttc gcttcattaa gaccatgatg atcctcttca atttgtcat 180
ctttctgtgt ggtgcagccc tgttggcagt gggcatctgg gtgtcaatcg atggggcaco 240
ctttctgaag atcttggggc cactgtctgc cagtgcctat cagtttgtca acgtgggcta 300
cttctctcat gcagccggcg ttgtggtctt tgccttgggt ttcttgggt gctatggtag 360
taagacggag agcaagtgtg cctcgtgac gttctctctc atctcctcc tcatcttcat 420

```

tgctgaagtt	gcagctgctg	tggtagcctt	gggtgacacc	acaaatgggtg	aaccttctct	480
gacgttctg	gtantgctg	ccatcaanaa	agattatggg	ttcccaggaa	aatctcactc	540
aanntatgaa	caacnccatg	aaaagggctc	caatttctgn	tggcttcccc	aactataccg	600
gaattttgaa	agantcccc	tacttccaaa	aaaaaanant	tgcctttccc	ccctttctgt	660
tgcattgaaa	acntcccaaa	acngccaatn	aaaactgccc	cnnccaaaaa	ggntcncaaa	720
caaaaaaant	nnaaggggtt					740

<210> 18
 <211> 802
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(802)
 <223> n = A,T,C or G

<400> 18						
ccgctgggttg	cgctgggtcca	gngnagccac	gaagcagctc	agcatacaca	gcctcaatca	60
caaggtcttc	cgctgcccgc	scattacgca	gggcaagagc	ctccagcaac	actgcctatg	120
ggatacactt	tacttttagca	gocagggtga	caactgagag	gtgtcgagag	ttattctctt	180
gagcctctgt	tagtgaggga	agattccggg	cttcagctaa	gtagtcagcg	tatgtcccat	240
aagcaaacac	tgtgagcagc	cggaaggtag	aggaagagtc	actctcagcc	agctctctaa	300
cattgggcat	gtccagcagc	ttccaaaaca	cgtagacacc	agngggcctcc	agcaactgat	360
ggatgagttg	ggccagcgct	gcccccttgg	cgaacttggc	taggagcaga	aattgctctt	420
ggttctgcgc	tgtaaccttc	acttcgcgac	tcataactgc	actgagttgt	ggggacttgg	480
gctcaggatg	tccagcgacg	tgtttccgcc	ccctenccta	atgacaccgn	ccanncaacc	540
gtcggctccc	gcccantgng	ttcgtctgnc	ctgggtcagg	gtctgctggc	cnctacttgc	600
aancttctgc	nggcccattg	aattcaacnc	accgggaactn	gtangatcca	ctnattctat	660
aaccggacgc	cacggcnant	ggaactccac	tcctntttnc	tttacttgag	ggttaaggtc	720
accccttncg	ttaccttggg	ccaaaacntn	ccntgtgtcg	anattgtnaa	tanggnccna	780
tnccanccnc	atangaagcc	ng				802

<210> 19
 <211> 731
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(731)
 <223> n = A,T,C or G

<400> 19						
cnaagcttcc	aggttaacggg	cggnaaanc	tgacccnagg	tancanaang	cagncngcgg	60
gagccacccg	tcacngggng	nggtctttat	nggagggggc	ggagccacat	cnctggacnt	120
catgacccca	actcccncnc	ncncantgca	gtgatgagtg	cagaactgaa	ggtnacgttg	180
caggaaacca	gancaaannc	tgtcccmte	caagtcggcn	nagggggcgg	ggctggccac	240
gncateent	cnagtgtctn	aaagcccnnc	cctgtctact	tgtttggaga	acngcnnga	300
catgcccagn	gttanataac	nggngagag	tnantttgoc	tctcccttcc	ggctggccan	360
cgngtntgct	tagnggacat	aacctgacta	cttaactgaa	cccnngaatc	tnccnccctt	420
ccactaagct	cagaacaaaa	aacttcgaca	ccactcaant	gtccactgnc	tgctcaagta	480
aagtgtaccc	catncccaat	gntgtctnga	ngctctgncc	tgcnttangt	tgggtccctg	540
gaagacctat	caattnaagc	tatgtttctg	actgcctctt	gtcccttgna	acaancnacc	600
cncnntoca	agggggggnc	ggcccccaat	cccccaacc	ntnaatttna	tttanccccc	660
ccnccnggpc	cgccctttta	cnancntcnn	nnacngggna	aaacennnagc	tttncccaac	720
nnaatccncc	t					731

10

<210> 20
 <211> 754
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(754)
 <223> n = A,T,C or G

<400> 20
 tttttttttt tttttttttt taasaaacccc ctccattnaa tgnaaaacttc cgaattgtc 60
 caacccccctc ntccaaatna ccttttcgg gngggggttc caaccccaan ttanntttgg 120
 annttaaatt aaatnttnt tggnggnna anccnaatgt nangaaagt nasccenta 180
 tnancttnaa tncctggaaa cngtngntt ccaaaaatnt ttaaccctta antccctcgg 240
 aaatagttna nggaaaacccc aantctctnt aaggttggtt gaaggntnaa tnaaaanccc 300
 naccaattgt tttngccac gctgaatta attggnttcc gntgttttcc nttaaaanaa 360
 ggnancccc ggttantaaa tccccccanc cccaattata coganntttt ttngaatgg 420
 gancccnccg gaattaacgg ggnnnntccc tnttgggggg cnggnncccc cccntcggg 480
 ggttngggnc aggnccnaat tgittaaggg tccgaaaaat cctccnagg aaaaaancic 540
 ccaggntgag nntnggggtt ncccccccc caggccccc ctcgnanagt tggggtttgg 600
 ggggctcggg atttntttc cctntttnc tcccccccc cngggganag aggttngnt 660
 tttgntennc ggccccnccn aagancttin coganntnaa ttaaatcent gctngggga 720
 agtcattgn agggntaaan ggccccctnn cggg 754

<210> 21
 <211> 755
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(755)
 <223> n = A,T,C or G

<400> 21
 atcancocat gaccocnaac nngggacenc tancocggnc nnsenacenc eggccnatca 60
 angtnaganc actncnatin natcaacccc caccnactac gcccncnanc cnacgccta 120
 nncanatncc actganagcg cgangtngan ngagaaact nataccanag ncccanacn 180
 ccagctgtcc nanaangcct nnnatacngg nnnatccaat ntgnancctc cnaagtatin 240
 nncnccnat gatitfecin anccgattac cctnccccc tancocctcc ccccaacna 300
 cgaaggcact ggnocnaagg nngcgnccc ccgtagntc cccncaagt cncncacctc 360
 aactcaneen nattaacngc ttcttgagta tcactcccg aatctcacc tactcaactc 420
 aaaaanaten gatacaaat atncaagcc tgnntatnac actntgaatg ggtctctatt 480
 ttagnngtcc ntnaancctc ctaataette cagtctncc tncaccaatt cnaangget 540
 ctttcngaca gcatntttt gtcccnntt ggttcttan ngaattgcc ttctngaac 600
 gggctentct tttccttcgg ttancctggg ttcnncoggc cagttattat ttccntttt 660
 aaattccncc cttttanttt tggctttna aacccccggc cttgaaaaag gccccctgg 720
 aaaaggttgt ttganaaaa tttttgttt gtcc 755

<210> 22
 <211> 849
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(849)

<223> n = A,T,C or G

<400> 22

tttttttttt	tttttangtg	tngtctgtgca	ggtagagggt	taetacaant	gtgaanacgt	60
acgtctngga	taangcgacc	cgantttctag	gaannccct	aaaatcanac	tgtgaagatn	120
atcctgnana	cggaanggtc	accggngat	nstgctaggg	tgaccctcc	cannncttn	180
cataactng	nggcctgtgc	caccaccttc	ggcgcccnng	ngnccgggco	cgggtcattn	240
gnnttaaccn	cactnngcna	ncggtttccn	ccccnnnng	accngggcga	tccgggggtnc	300
tctgtcttcc	cctgnagncn	anasantggg	ccacggnccc	ctttaccct	nnacaagcca	360
cngcctctc	ncnnggccc	ccctccant	angggggact	gcnnanngt	cgtttactng	420
nnacccnnn	gggtacctcg	gttgctcgant	cnaccgnang	ccanggttc	cnaaggaag	480
tgcgttnttg	gcccctaccc	tctcctnccg	nnaccccttc	ccgaacnaga	ncgctcccg	540
cncnncng	cctnccctcg	caacacccgc	ctctctngt	ncggnnacc	cccccccg	600
ncctctcnc	ngnngnanc	ctcccccnc	gtctcannca	ccaccccgcc	ccgcacggcc	660
ntcancnnc	gngngacnng	nagncnntc	gncgcgcgc	gggnncnct	cgcncngaa	720
ctcctctngg	ccantnncgc	tcannccnna	cnaaacgcgc	ctgcgcggcc	cgnagcgacc	780
ncctccacga	gtctcccg	cttcncccc	angnttcca	cgaggacacn	nnaccccgcc	840
nnccangcg						849

<210> 23

<211> 872

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(872)

<223> n = A,T,C or G

<400> 23

ggcgaacta	tactctgtc	gnactctgtc	gcctcgtcnc	tcttttctc	cgcacccatg	60
tctgacnanc	cagattaggg	ngatctcnan	agntctganc	agtcacaaat	gantaacaca	120
cacacnncn	aganaaatcc	netgccttcc	anagtanaen	attgaacnng	agaaaccangc	180
nggcgaatcg	taatnagggc	tgcgcgcgca	atntgtcncc	gtttatttn	ccagctcnc	240
ctnccnacc	tactctctcn	naetgtctcn	ccccctngtn	cgnaccccc	aggtctggga	300
tccgggtttn	antgacggng	cnnccctcc	ccccctccat	nacganccac	cgcacccacc	360
nanngcncc	cccccgncct	cttcgcaccc	ctgtctcttn	ccccctgtngc	ctggcnncngn	420
accgcattga	cctctgcann	ctnccngaaa	ncgnanacgt	ccgggttggn	annanogctg	480
tgggnanccg	tctgcncgc	gttccctccn	nenncttcca	ccatcttct	tacngggctc	540
ccnccgctc	tannacacn	cctgggagcc	ntctctntgc	cccccttnac	tccccccctt	600
cgnctgtgac	cgncccccac	ntcatttnca	naegntcttc	acaaanncc	ggntnnctcc	660
cnanncngcn	gtcannccag	ggaagggngg	ggnnccnntg	attgacgttg	aggnngangtc	720
cgaanantcc	tcnccctcan	cctacccct	cggcggnact	ctcngttnc	aacttancaa	780
ntctccaccg	ngnccnctc	tcagctcnc	ccccccnct	ctctgcantg	tactctgtc	840
tnaccnntac	gantttctgn	cncctctttt	cc			872

<210> 24

<211> 815

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(815)

<223> n = A,T,C or G

<400> 24

gcattgcaagc	ttgagtattc	tatagngtca	cctaaatanc	ttggcntaat	catggtcnta	60
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netgnetttcc tgggtcaaat gtatacnaaa tanatatgaa tctnatntga caagannqta 120
tcnfnccatta gtaacaantg tnnatgtccat cctgtcngan canattccca tnnattncgn 180
cgcattoncn gnccantain taataggga stonnnntnan ncaccnscat ctatcstcc 240
gcnccctgac tggagagat ggaatnattc tnnatgtacc nactgttca tcttgattn 300
aanaccccc cgcngnccac cgggtagnng cnagccnntc ccaagacctc ctgtggaggt 360
aacctggctc agannccatc aacntgggaa acccgcncc angtnnaagt ggnnnccan 420
gateccgtcc aggnitnacc atcccttcc agcgccctt tngtgcctt anagnnagc 480
gtgtccnanc cnetcaaat ganacggcc agnccancc caattnggca caatgtcgc 540
gaacccccca gggggantna tncancccc caggattgtc cnccnagaa atccnccan 600
ccccccctac cccnctttgg gacngtgacc aantcccgga gtaccagtc ggcngnctc 660
ccccacgggt naccntgggg ggggaaact cngnntcanc cngnccgggn stcgnaaaga 720
accggncctn ggcggaaag ancnntcng agncccnct cgtataccc cccctcncca 780
ccnaacngnt agtccccc cngggtacgg aagg 815

```

<210> 25
 <211> 775
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(775)
 <223> n = A,T,C or G

```

<400> 25
ccgagatgtc tggctccgtg gccttagctg tggctcggct acctctctctt tctggcctgg 60
aggtatccca ggtactcca aagattccgg tttactcagc tcattccagca gagaatggaa 120
agtcaaatct cctgaattgc tatgtgtctg ggtttcctcc atccgacatt gaanttgact 180
tactgaagaa tgganagaga attgaaaaag tggagcattc agacttgtct ttcagcaagg 240
actgggtctt ctatctcttg tactacactg aattcaccac cactgaaaa gatgagtatg 300
cctggcgtgt gaaccatgtg actttgtcac agcccaagat agttaagtgg gatcgagaca 360
tgtaaagcag cncatcgaa gtttgaaagt gccgcatttg gattggatga attccaaatt 420
ctgcttgctt gcttttcaat antgatgtgc ntatacacc taccctttat gnccccaat 480
tgtgggggtt acatnattgt tcnctnagga catgatcttc ctttataant cncncttgc 540
aatgccegt cncnctttn ngaatgttgc cnaaccacg gtgggtccc ccaggtcncc 600
tcttaaggaa gggcctgggc cnetttncaa ggttggggga accnaaatt tcncttntgc 660
ccncccncca cmtcttgng nnnccanttt ggaacccttc cnattccctt tggcctcnna 720
nctttncta aaaaaacttn aaanctngc naanatttn acttccccc ttacc 775

```

<210> 26
 <211> 820
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(820)
 <223> n = A,T,C or G

```

<400> 26
anattantac agtgaatct tttccagag gtgtgtanag ggaacggggc ctagagccat 60
ccanagata ncttatnca acagtgtttt gaccaagagc tgcctgggac atttctgca 120
gaaaaggtgg cgtcccccac cactctctct ctcccatagc catccagag gggtagtag 180
ccatcangcc ttccgtggga gggagtcang gaacaacan accacagagc anacagacca 240
ntgatgacca tggcggggag cagacctctt cctgnaccg ggggtggcna nganagccta 300
netgaggggt cacactataa acgttaacga ccnagatnan cactgtcttc aagtgcacc 360
ttctacctg acnaccagng accnnnaact gcngcctggg gacgcnctg gganacgcta 420
acnnagcaet cccctgccc cccatggccg tccgntccc tggctctgnc aagggaagct 480

```

```

ccctgttgga attncgggga naccaaggga nccccctctt ccancgttga agggaaaaan 540
gatgggaattt tnccttccg gccnntcccc tcttcttita cagccccctt nntactcttc 600
tccctctntt nctctgnenc acttttnacc ccannatttc ccttnattga tcggannctn 660
ganattccac tncgcctnc cctcnateng naanacnaaa nactntctna ccnnggggat 720
gggnaectcg nctactctt ctttttenct accnccnntt ctttgcctct ccttngatca 780
tccaaacntc gntggccntn ccccccnnt testttnccc 820

```

```

<210> 27
<211> 818
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(818)
<223> n = A,T,C or G

```

```

<400> 27
tctgggtgat ggcctcttcc tctcaggga cctctgactg ctctgggcca agaatctct 60
tgtttcttct ccgagcccca ggcagcggtg attcagccct gcccaacctg attctgatga 120
ctggggatgc tgtgacggac ccagggggca aatagggtcc caggggtccg ggagggggcg 180
ctgctgagca ctccggcccc tcacccctgcc cagcccttgc catgagctct gggtctggtc 240
tcggcctcca gggttctgct ctccangca ngccancaag tgggcctggg ccacactggc 300
ttcttctctg cccntccctg gctctganc tctgtcttcc tgtctgtgct angcncctg 360
gatctcagtt tccctenctc anngaactct gttctgann tcttcantta actatgaatt 420
tatnacnna tggnetgtnc tgtcnnaactt taatgggccc gacgggctaa tccctccctc 480
nctccctcc anttcnana accngcttnc cntctctcc ccntanccg ccnggggaac 540
ctcctttgcc ctnacccang gccnnaaccg ccctnnctn gggggggcng gtanctnnc 600
ctgntnacc cncfchcnnt tncctctgct cncnncngc angcannctc ncngtccnna 660
tncctcttcc ngntnctnaa ngntcnctn tnnnnngnc ngntnctnna tccctctcnc 720
cnnntgnang tnnntnnnc ncngncccc nnnnnnnna nggnntnna tctnchngc 780
ccnncccccc ngnattaagg cctccnctct ccggccnc 818

```

```

<210> 28
<211> 731
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(731)
<223> n = A,T,C or G

```

```

<400> 28
aggaaggcg gagggatatt gtangggatt gagggatagg agnataangg gggaggtctg 60
tcccaacatg anggtgngt tctcttttga angagggttg agtttttann ccnggtcgg 120
gattnaaccc cttgttatgg agnnasaggn tttnagggat ttttcggctc ttatcagtat 180
ntanattcct gtnaatcgga aaatnatntt tcnnnggaa aatnttctc ccctccgnaa 240
atttctccg ggtagtgcct nttinggggn ccgccangtt tcccaggctg ctanaatcgt 300
actaaagntt naagtggan tcaaatgaa aacctnccac agagnatccn taccgactg 360
tannttncct tgcctctntg acctctgcnng agcccaatac ccnngngnat gtcncccngn 420
nnngcgnnc tgaaannnnc tcngggctnn gancatcang gggtttctga tcaaaagcnn 480
cgttttncat naaggcactt tagctcctc caaccnctng cctcnncce tttngcctc 540
nggttncct acgtntntng cncctnntn ganattttnc ccgctctggg naancctct 600
gnaatgggta gggctctntc ttttnacnn gggtntact aatcnctnc accntnctt 660
tctcnacccc cccctttt tcttccncc ggnaatggg gtctcccnna ccngggggg 720
nnccccnnc c
731

```

14

<210> 29
 <211> 822
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(822)
 <223> n = A,T,C or G

<400> 29
 acagtcacag tgggtggaa ttccattgtg ttgggggncnc ttctatgant antnttagat 60
 ogctcaanacc tcacancctc ccnacnangc ctataangaa nannaataga nctgtncant 120
 atntntacnc tsatanncct cnnnaccacac tccctcttaa cccnactgtt gcttatngcn 180
 tnnctantct ntgcgcctn cnanccacn gtgggcnac cncnagnatt ctenatctoc 240
 tcnccatntn gcttananta ngtnccatcc ctatacctac nccaatgcta nnnctaanen 300
 tccatnantt annstaacta cccctgcctt ngactttcnc atnanctoct aatttgaatc 360
 tactctgact cccacngcct annnattagc anctccccc nactnatctt caaccacatc 420
 ntccacaccc tatctanctg ttncnccacc nttnccctcc atcccccnnac aacccccctc 480
 ccaaataccn nccacctgac ncttaacccn caccatcccg gcaagccnan ggnctattan 540
 cccctgggaa cscnatngga nsaasaaaac ccaactctc tancnennat ctccctaana 600
 aatnctctn naatttactn ncsntnccat caanccacn tgaaacnnaa cccctgtttt 660
 tanatccctt ctttogaasa cnaacccctt ananccccc ctttnggggc cccccactnc 720
 ccaaatgaag gncncccaat cnaagaaacy nccntgaasa encnaggcna anannatccg 780
 canatccctat cctttanttn ggggnccctt nccnggggce cc 822

<210> 30
 <211> 787
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(787)
 <223> n = A,T,C or G

<400> 30
 eggcgccttg ctctggcaca tgcctcctga atggcatcaa aagtgatgga ctgcccattg 60
 ctagagagga ccttctctcc tactgtcatt atggagccct gcagactgag ggctcccctt 120
 gtctgcagga tttgatgtct gaagtctgtg agtgtggctt gtagctctct atctacatna 180
 gctggaagcc ctggagggcc tctctcgcca gctccccct tctctccag ctctccangg 240
 acaccagggg ctccaggcag cccattatc ccagnangac atgggtgtttc tccagcgagg 300
 cccctggggc ctgnaaggcc aggtctctct ttgacacct ctctccgctc ctgctcgga 360
 ggccttgga tccactantt ctanaacggg cgcacccnag gtgggagctc cagcttttgt 420
 tccnttaast gaaggttaast tgcncgctt gcttaatcat nggtcanaac tntttctgt 480
 gtgaattgt ttntccctc ncnattccnc ncnacatacn aacccgggan cctaaagtgt 540
 taaagcctgg gggtnccctn nngaatnaac tnaactaat taattgcgtt ggctcatgsc 600
 ccgctttccn ttonggaaaa ctgtctctcc ctgcttntat gaatcgcca cccccnggg 660
 aaaagcggtt tgcnttttng ggggntccct cnccttccc cctcnctaan cctnccgct 720
 cggctgttnc nggtngcggg gaanggggat nnnctccnc naagggggng agnangtat 780
 ccccaaa 787

<210> 31
 <211> 799
 <212> DNA
 <213> Homo sapien

<220>

15

<221> misc_feature
 <222> (1)... (799)
 <223> n = A,T,C or G

<400> 31
 tttttttttt ttttttttgc gatgctaactg ttttaattgca ggaggtgggg gtgtgtgtac 60
 catgtaccag ggctattaga agcaagaagg aaggaggagg ggcagagggc cctgctgagc 120
 aacaaaggac tcttgcagcc ttctctgtct gtctcttggc gcaggcacct ggggaggcct 180
 cccgcagggt gggggccacc agtccagggg tgggagcaat acanggggtg ggaagtgggtg 240
 gtggtggtn cnaatggcct gncacaaatc cctacgattc ttgacacctg gatttcacca 300
 ggggaacctc tgttctccca agynaacttc ntnnatctcn aaagaacaca actgtttctt 360
 cngcanttct ggctgttcat gaaaagcaca gggtgcctat ttnggctggg acttggtaca 420
 tatgtttccg gcccacctct cccctcnaaa aagtaattca ccccccccn cctctcttgg 480
 cctgggacct taantacca caccgggaact canttanita tctctcttng gntgggtctg 540
 ntnatcccn cctgaangcg ccaagttgaa aggcacagcc gtcccctc cccatagnan 600
 nttttnaant canctaactg cccccnnggc aacnatacaa tcccccccn tggggggccc 660
 agcccnnggc ccccgctctg ggnnccngn cncgnantcc ccaggntctc ccantcngnc 720
 ccnnngncc cccgcacgca gaacanaagg ntngagccnc cgcannnnnn aggtncnca 780
 ctcgcccccc cccnngnng

<210> 32
 <211> 789
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)... (789)
 <223> n = A,T,C or G

<400> 32
 tttttttttt tttttttttt tttttttttt tttttttttt tttttttttt 60
 ttttaccnag ggcaggttta ttgacaacct cncgggacac aancaggctg gggacaggac 120
 ggcacagggc tccggcgggc gcyggcgggc cctacactgc ggtaccnaat ntgcagctc 180
 cgtctccgtt tgatatctct ctgcagctgc aggatgcctt aaaaacaggc ctcggcctn 240
 ggtggggcacc ctgggatttn aatttccacg ggcacaatgc ggtcgcanc cctcaccacc 300
 nattaggaat agtggintta cccnccnccg ttggcncact ccccttggaa accacttntc 360
 gcggtccggc catctggtct taaaccttgc aaacnctggg gccctctttt tggttantst 420
 nccngccaca atcatnacte agactggcnc gggctggccc caaaaaaann ccccaaaacc 480
 ggcacatgic ttncgggggt tgcctgcnaa tncatcacct cccggggcna ncaggncac 540
 ccaaaagtgc ttgngggccn caaaaaaant ccgggggggnc ccagtttcaa caaagtcatc 600
 ccccttggcc cccaaatcct cccccgntt nctgggtttg ggaacccag cctctnctt 660
 tggnaaggcaa gntggntccc ccttcggggc ccgggtgggc cccnctctsa ngaaaaamcc 720
 ntctnnnca ccatcccccc nagnnacgnc tancangna tccctttttt tanaaacggg 780
 cccccnng

<210> 33
 <211> 793
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)... (793)
 <223> n = A,T,C or G

<400> 33
 gacagaacct gttggatggt ggagcaacctt tctatacgac ttacaggaca gcagatgggg 60

```

aattcatgac ttttggagca atanaacccc agttctacga gctgctgac aaaggacttg 120
gactaaagtc tgatgaactt cccaatcaga tgagcatgga tgattggcca gaaatgaana 180
agaagtttgc agatgtattt gaaaagaaga cgaaggcaga gtggtgtcaa atctttgacg 240
gcacagatgc ctgtgtgact cgggtttctga cttttgagga ggttgttcat catgatccca 300
acaangaaag gggctcgttt atcaccantg aggagcagga cgtgagcccc cggcctgcac 360
ctctgctggt aaacaccccc gccatccctt ctttcaaaag ggtaccacta cttctagagc 420
ggncggccac ggggtggagc tccagctttt gttcccttta gtgagggtta attgocgct 480
tgggcgaatc atggctatan ctgtttctgt tgtgaattg ttatccgctc acaattccac 540
acaacatacg anccggaagc atnaaatttt aaagcctggg ggtngcctaa tgantgaact 600
naetccactt aattggcttt gcgctcactg cccgctttcc agtcgggaaa acctgtcctt 660
gccagctgcc nttaatgaat cgggccaccc cccggggaaa agcngtttg cttnttgggg 720
cgnccttccc gctttctcgc tteetgaant ccttccccc ggtctttcgg cttggggcna 780
acggtatcna cct 793

```

```

<210> 34
<211> 756
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(756)
<223> n = A,T,C or G

```

```

<400> 34
gcgcgagccg gcattgacga gcaactcag ggcgagtga accgtaaaag ccccaatctt 60
ancaagtgcg ggggaanagc gggtagactc aagctagttc ttctggagct caacttcttg 120
ccaaaccacag ggaaccaagc gaccasaacg cagctaatte tggcccgtag cactactggag 180
atcggggccc aatggagcat cctacgcaan gacatccctt ccttcgagcg ctacatggcc 240
cagctcaaat gctactactt tgattacaan gacgagctcc ccgagtcage ctatatgcac 300
cagctcttgg gcctcaacct cctcttcttg ctgtcccgga accgggtggc tgantnccac 360
acgganttgg ancggtctgc tgcaccaaga catcacaccc aatgtctaca tcnaccacca 420
gtgtccttga gcaatactga tgganggcag ctacencaaa gtttctcttg ccnagggtta 480
catcccccgc cgagagctac accittctca ttgacatcct gctcgacact atcagggttg 540
aaaatcgccg ggttgcctca gaaaggctac aanaanctcc ttttctctga aggcocccgg 600
atnctnctgt nctagaatcg gcccgcacat gcggtgganc ctccacctt tcttctcct 660
ttactgaggg ttatttgcg cccttgcggt tatcatggtc aenccngttn cctgtgttga 720
aatntttaac ccccacaaat tccagccna caitng 756

```

```

<210> 35
<211> 834
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(834)
<223> n = A,T,C or G

```

```

<400> 35
gggatctct anactnaact gnatgcattg ttgtcgtgt ggtcgtgtc gatgaanag 60
aacaggatct tgccttgaa gctctcggct gctgtnttta agttgctcag tctgcctga 120
tagtcagaca cnetcttggg caaaaaaan caggatntga gtcttgattt caccctccat 180
aatcttcngg gctgtctgct cgggtgaact gatgacnang ggcagctggt tgtgtntgat 240
aaantccanc angttctcct tggtagacct ccttccaaag ttgttcgggc cttcatcaaa 300
cttctnnaan angannance canctttgtc gacgtggnat ttgganacca cgtcactgtt 360
ggaaactgat cccaaatggt atgtcatcca tgcctctctg tgcctgcasa aaacttgctt 420
ggncaaate cgaactcccc tccctgaag agccnatac cccccctc cctggactcc 480

```

```

ncaangact ctcccgctac cccntccnag caggggttggg ggcannocgg gcccntgcgc 540
ttcttcagcc agttcaenat ttctcatcag cctcttgcca gctgttntat tccctggggg 600
ggaancgctc tctcccttcc tgaannaact ttgacogtng gaatagccgc gentcncnt 660
acntnctggg ccgggttcaa antccctccn ttgnmntcn cctcgggcca ttctggattt 720
noonaacttt ttcttccccc cncocncgg ngtttgntt ttcaalnggg ccccaactct 780
gctnttggcc antccctgg gggcntntan cccccctnt ggtccntng ggc 834

```

<210> 36
 <211> 814
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(814)
 <223> n = A,T,C or G

```

<400> 36
oggnccgttt ccccgccgc cccgtttcca tgaacnaagg tcccttcang ttaaatacnn 60
cctagnaaac attaatgggt tctctacta atacatcata cnaaccagta agcctgccc 120
naacgccaac tcaggccatt cctaccaaag gaagaaggc tggctctctc ccccccgtga 180
ggaaggccct gcttgtaag acaccacaat ccggctgaat ctnaagtctt gtgttttact 240
aatggaaaaa aaaaataaac aaaggtttt gttctcatgg ctgccacccg cagcctggca 300
ctaaaaacac ccagcgctca ctctcgcttg gaaaaatatt ctttgctctt ttggacatca 360
ggcttgatgg tctactgac acctttccac ccagctgggc ccccttcccc catntttgtc 420
antganctgg aaggccgtga ncttagtctc caaaagtctc ngcccacaag accggccccc 480
aggggaagtc ntttccagtg gactgcacaa anataccccc tatcatcnnl gaataaaaaag 540
gccctgaac ganatgttc caccanccct taagaccat aatcctngaa ccctgggtgc 600
cttccggtct gatccnaaag gaatgttctt gggccccant cctcctttg ttncctacgt 660
tgtnttggac cctgtctngn atnaccaan tganatcccc ngaagcaccc tccccctggc 720
atttganttt cntaattct ctgccctacn nctgaagaca cnattccctn ggncccnaaa 780
ggngaactca agaaggtctn ngaaaaacca cncn 814

```

<210> 37
 <211> 760
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(760)
 <223> n = A,T,C or G

```

<400> 37
gcctgctgct ctctctcaaa gttgttcttg ttgccataac aaccaccata ggtaaagcgg 60
gcgcagtggt cgttgaaggg ttgttagtac cagcgcggga tctctctctt gcagagtcct 120
gtgtctggca ggtccacgca atgccccttg tcaatgggga aatggatgag ctggagctcg 180
tcnaanccac tctgttatt ttccacngca gctctctccg aagcttccgg gcagttgggg 240
gtgtctgtac actccactaa actgtcgatn caccagccca ttgctgcage ggaactgggt 300
gggtgacag gtgccagaac acactggatn ggcctttcca tggaaaggcc tgggggaaat 360
cncctnancr caaactgect ctcaaaaggc accttgaca ccccgacagg ctagaastgc 420
actcttcttc ccaaggtag ttgttcttgt tgcacaagca ncctccacca aaccnaaenc 480
ttgcasaatc tctccgtgg ggtcatann taccanggtt ggggaanaaa acccgccngn 540
gancncctt gtttgaatgc naaggnaata ctctctctgt cttgcttggg tggaaagca 600
caattgaact gttaacnttg ggcgggttc cctnnggtg gtctgaaact aatcaccgte 660
actggaaaaa ggtangtgc ttcttgaat tcccaantt cccctngntt tgggtnttt 720
ctctctncc ctaaaaatcg tnttccccc cctangggg 760

```


<210> 38
 <211> 724
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(724)
 <223> n = A,T,C or G

<400> 38
 tttttttttt tttttttttt tttttttttt tttttttttt cccctccat tgaatgaaaa 60
 ettccaaat tgtccaaacc cctcnnccaa atnccattt cggggggggg gtccaaacc 120
 caatttaatt ttgganttta aattsaatnt tnattggggg aanaaaccaa atgtnaagaa 180
 aatttaaccc attatnaact taaatnccn gaaacccntg gnttccaaaa atttttaacc 240
 cttaaatccc tccgaattg ntaanggaaa cccaaaiten cctaaggctn tttgaaggtt 300
 ngatttaaac ccccttnant ttttttnacc cnagncnaa statttngnt tccggtgttt 360
 tectnttaan cntaggtaac tcccgntaat gaannccot aanceaatia aaccgaattt 420
 tttttgaatt ggaattccn aggggaattna cgggggtttt tcccttttg gggccatncc 480
 cccncttctg ggttttggg ataggttgaa ttttttnang ncccaaaaaa ncccccaana 540
 aaaaaactcc caagnnttaa ttngaantc ccccttccca ggccttttg gaaaggggg 600
 tttntggggg cccgggaatt cnttccccc ttccccccc ccccccnggt aaagggttat 660
 ngnttttgtt ttttggggcc cttnanggae cttccggatn gaaattaat ccccggnccg 720
 gccg 724

<210> 39
 <211> 751
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(751)
 <223> n = A,T,C or G

<400> 39
 tttttttttt tttttttttg ctccatttta atttttattt tgattttttt taatgctgca 60
 caacacaata tttatttcat ttgtttcttt tttttcattt tttttgtttg ctgctgctgt 120
 tttatttatt tttactgaaa gtgagaggga acttttcttg ccttttttcc tttttctgta 180
 ggcgccttta agctttctaa atttgaaca tctaagcaag ctgaanggaa aagggggttt 240
 cgcaaaatca ctggggggaa aggaagggtt gctttgttaa tcatgcocta tgggtgggtg 300
 ttaactgctt gtacaattac atttcaattt taattsaattg tgcctaaagc ttttaattana 360
 cttgggggtt cctccccc anccaccccn ctgacaaaaa gtgcncgccc tcaaatnatg 420
 tccggcnnnt cnttgaaca caacngcnaa ngttctcatt ntcccnenc caggtnaaaa 480
 tgaagggtta ccatntttaa cncacccctc acntggcnnn gctgaatcc tcnaaaancn 540
 cctcaancn aattctnng ccccggtenc gctnngtcc cncccggct cgggaantn 600
 cccccccga annccntnnc naccnaaatt ccgaaatat tcccnctnc tcaattcccc 660
 cnnagactnt cctcnnncn cncatttttc tttntatcac gaacncgnc cnaaaatgn 720
 nnnncnctc cncnngtcn naatncncn c 751

<210> 40
 <211> 753
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(753)